We present an interesting case of a double gene hit; with pathogenic mutations in HESX1 and GLI2, and explore the possibility of pituitary gene interactions in the phenotypic differences. The role of GLI2 and HESX1 in the phenotypes of the two siblings is unclear as these variants have yet to be fully characterised. Whilst these two variants were shown to be pathogenic.

HESX1 (homeobox expressed in ES cells 1)
- HESX1 is a homeobox-containing transcriptional repressor crucial for the initial determination and differentiation of the pituitary gland and development of the forebrain.
- Mutations in HESX1 have been linked to a variety of phenotypes ranging from IGHD to Combined Pituitary Hormone Deficiency (CPHD) to Septo-Optic Dysplasia (SOD),
- SOD is a highly heterogeneous condition with multi-factorial aetiology, and HESX1 causes SOD by oligogenic interaction. However, there is no clear genotype-phenotype correlation thus far, and variable penetrance.
- The paternally derived c.475C>T mutation is only seen in patient K, and likely to be a pathogenic variant.

GLI2 (GLI family zinc finger 2)
- GLI2 is a zinc-finger transcription factor involved in the Sonic Hedgehog (Shh) signalling pathway, implicated in brain and pituitary embryogenesis.
- GLI2 is a large, highly polymorphic gene. Various rare mutations have been detected in individuals with a spectrum of clinical phenotypes, ranging from frank holoprosencephaly (HPE), craniofacial abnormalities, polydactyly, panhypopituitarism, secondary hypogonadism, as well as IGHD.
- The maternally derived c.2671dupG nucleotide change seen in our patients, is predicted to result in a prematurely truncated GLI2 protein (Ala891Gys*140) and has been classified as a pathogenic variant.

Discussion
- We present an interesting case of a double gene hit; with pathogenic mutations in HESX1 and GLI2, and explore the possibility of pituitary gene interactions in the phenotypic differences.
- Patient K possesses both the pathogenic GLI2 variant and HESX1 variant and appears to have a more severe phenotype than his brother who only carries the GLI2 variant. This raises the possibility of an interaction between the two pathogenic gene variants in contributing to patient K’s phenotype. However, the role of other environmental factors cannot be excluded.
- Our case is consistent with the literature, demonstrating the variable penetrance of GLI2 and HESX1, as well as the variable expressivity.
- The role of GLI2 and HESX1 in the phenotypes of the two siblings is unclear as these variants have yet to be fully characterised. Whilst these two mutations have been reported before and linked to the spectrum of phenotypic presentations, this is the first report in the literature of a ‘double-gene hit’ in familial hypopituitarism.

We report varying clinical presentation of IGHD in two siblings from a non-consanguineous family. Both presented with failure to thrive, mid-facial hypoplasia and were found to have low IGF-1, IGHD and MRI findings of severe pituitary hypoplasia and Chiari 1 malformation.

Genetic analysis revealed both siblings had a maternally derived GLI2 variant, and the older brother had an additional paternally derived HESX1 variant. Both variants were shown to be pathogenic.

Patient K: At birth Patient K was noted to have bilateral cryptorchidism and left foot postaxial polydactyly.
- In the initial neonatal period, he suffered from initial poor temperature regulation, brittle glycaemic control and poor weight gain.
- He had mild gross motor developmental delay.
- Just before the age of 4, he was found to have growth hormone deficiency and was started on replacement GH therapy.
- 2 months later, MRI findings showed a hypoplastic pituitary and a chiari1 malformation.
- Whole exome sequencing age 6 found a homozygous maternal OFD1 variant (insert variant) of unknown significance.
- Following, the 100,000 genome study related to GH deficiency found a homozygous maternally derived OFD1 variant, and the older brother had an additional paternally derived HESX1 variant. Both variants were shown to be pathogenic.

Patient N: At birth Patient N was noted to have frontal bossing and midfacial hypoplasia but of a milder phenotype than his brother. He also had normal genitalia with bilaterally descended testes.
- Glucagon stimulation testing was performed, showing a GH deficiency.
- Subsequent MRI of the pituitary showed severe pituitary hypoplasia and a chiari1 malformation.
- Genetic analysis revealed only a pathogenic GLI2 variant.

We report interesting Genotype-Phenotype Differences in Siblings with Familial hypopituitarism and Pituitary Hypoplasia