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CONGENITAL HYPOPITUITARISM: NOVEL PHENOTYPES, NOVEL MECHANISMS

Mehul Dattani

UCL GOS Institute of Child Health London/
Great Ormond Street Children's Hospital, London

DISCLOSURES

- Grants/Honoraria from NovoNordisk and Sandoz

CONGENITAL HYPOPITUITARISM (CH)

- Incidence 1 in 4,000 – 1 in 10,000 births
- Early neonatal presentation e.g. low blood glucose or later with growth failure
- May be single e.g. GHD or multiple (MPHD/CPHD)
- Can evolve to include other hormonal deficiencies
- Associated abnormalities of eyes, ears, other parts of forebrain, palate

CH, congenital hypopituitarism; CPHD, combined pituitary hormone deficiency; GHD, growth-hormone deficiency; MPHD, multiple pituitary hormone deficiency

SEPTO-OPTIC DYSPLASIA DEFINITION

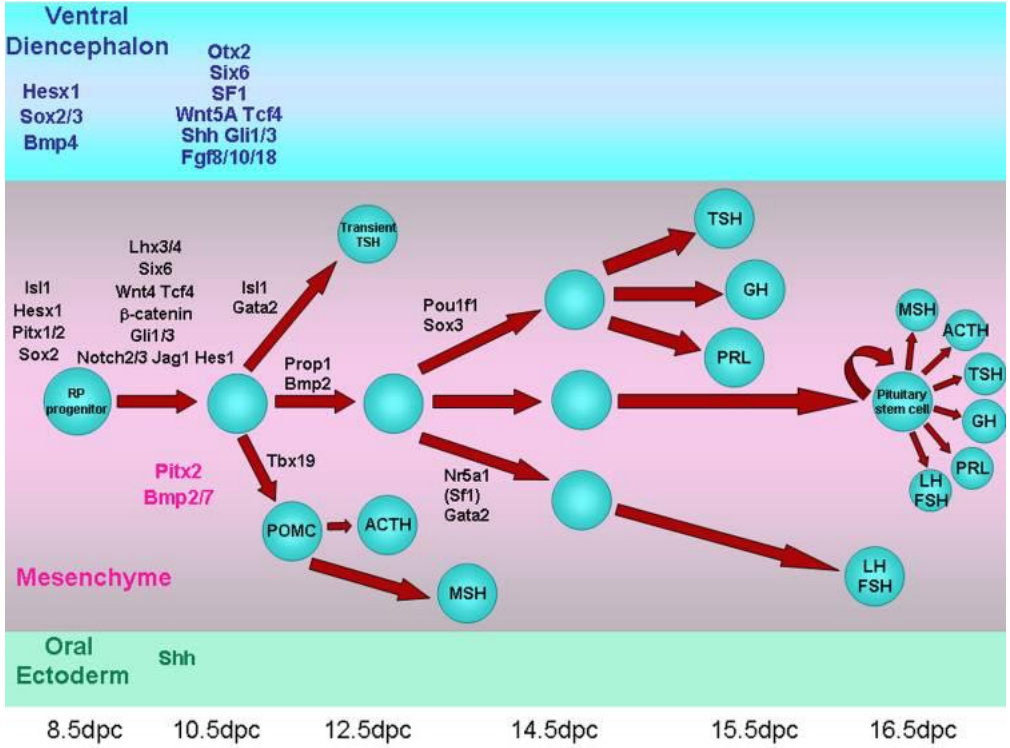
- Described by Reeves in 1941 – absence of septum pellucidum associated with optic nerve problems
- Variable combination of midline forebrain abnormalities, eye abnormalities and pituitary abnormalities[1,2]
  - 2/3 features to make the diagnosis
- Rare: reported incidence 1/10,000
- Commoner in younger mothers: controversial
- Mean age of mothers[3]
  - SOD: 25.1 years (n=113)
  - CPHD: 29 years (n=117)

CPHD, combined pituitary hormone deficiency; SOD, septo-optic dysplasia

1. Patel L, et al. J Pediatr. 2006;148:85-8. 2. Webb EA, et al. Eur J Hum Genet 2010;18:393-7. 3. McNay DE, et al. J Clin Endocrinol Metab. 2007;92:691-7

GENETIC CASCADE IN PITUITARY DEVELOPMENT

- Signaling molecules
  - E.g. FGF, BMP4, Wnt, Notch, Shh
  - Widely expressed
- Transcription factors
  - Homeobox genes, HMG family, TBX family
  - Regulate gene expression; activators and repressors



BMP4, bone morphogenetic protein 4; FGF, fibroblast growth factor; HMG, high mobility group; SHH, sonic hedgehog; TBX, T-box transcription factor; WNT, wingless-type MMTV integration site family

Kelberman D, et al. Endocr Rev. 2009;30:790-829

STRATEGIES FOR GENE IDENTIFICATION

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Candidate gene approach

- Mouse models
  - Naturally-occurring
  - Transgenic
- Chromosomal abnormalities
- Genome Mapping Strategies
  - Linkage analysis
  - Comparative Genomic Hybridization
  - SNP arrays

Next generation sequencing

- Whole exome sequencing
- Whole genome sequencing

SNP, single-nucleotide polymorphism

GENES CURRENTLY IMPLICATED IN CONGENITAL HYPOPITUITARISM

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Early genes	Genes implicated in cellular differentiation	Genes leading to IGHD
• SOX family: *SOX2*, *SOX3* • *HESX1* • *OTX2* • LIM family: *LHX3*, *LHX4* • *GLI2* • *TCF3/TCF7L1* • KS genes • *FOXA2* • *ARNT2* • *IGSF1* • *PNPLA6*	• *PROP1* • *POU1F1* • *KCNQ1*	• *GHRHR* • *GH1* • *RNPC3*

IGHD, isolated growth hormone deficiency; KS, Kallmann syndrome

Adapted from Gregory LC, Dattani MT. J Clin Endocrinol Metab. Epub Nov 8, 2019

BACKGROUND

Congenital hypopituitarism disorder	Incidence	Description	Known candidate genes
Multiple pituitary hormone deficiency (MPHD) without midline defects	1/4000	Deficiencies in one or more of the 6 anterior pituitary hormones: GH, TSH, LH, FSH, PRL, ACTH	*HESX1, SOX3, GLI2, LHX3, LHX4, PROP1, POU1F1, KAL1, PROKR2, PNPLA6*
Septo-optic dysplasia (SOD)	1/10,000	Optic nerve hypoplasia (ONH), Midline neuroradiological abnormalities. Pituitary hypoplasia - consequent endocrine deficits	*SOX2, OTX2* *HESX1* *PROKR2, FGF8* *KAL1* *TCF7L1*
Holoprosencephaly	1/10,000 - 1/20,000	Incomplete cleavage of the prosencephalon, affecting both the forebrain and the face: Alobar (no forebrain division) Semilobar (some separation) Lobar (complete separation) Microcephaly, hypotelorism, a single central maxillary incisor, cleft lip and/or palate	*SHH* *GLI2* *ZIC2* *SIX3* *TGIF1* *FGF8* etc. Sub-microscopic deletions at a number of loci

ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; MPHD, multiple pituitary hormone deficiency; ONH, optic nerve hypoplasia; PRL, prolactin; SOD, septo-optic dysplasia; TSH, thyroid-stimulating hormone

Adapted from Gregory LC, Dattani MT. J Clin Endocrinol Metab. Epub Nov 8, 2019. Fang Q, et al. Endocr Rev. 2016;37:636-75

BACKGROUND

Congenital hypopituitarism disorder	Incidence	Description	Known candidate genes
Hypogonadotropic hypogonadism (HH)/ Kallmann syndrome (KS)	Males: 1/10,000 Females: 1/50,000	Failure to activate pulsatile secretion of GnRH, causing deficiencies in LH, FSH. Delay in onset/complete/partial failure of puberty Anosmia	*GnRHR* *KAL1* *PROK2* *PROKR2* *FGF8* *FGFR1 etc*
Isolated pituitary hormone deficiency (IGHD)	1/4,000 – 1/10,000	The most common isolated deficiency - short stature, delayed growth velocity and skeletal maturation	*GH1, GHRHR, RNPC3* *HESX1, OTX2* *SOX3* *POU1F1*
Isolated TSH deficiency	1/20,000 – 1/80,000	–	*TSHβ, TRHR, TBL1X, IGSF1*
Isolated ACTH deficiency		Neonatal hypoglycaemia	*TBX19 (TPIT), POMC*

ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HH, hypogonadotropic hypogonadism; IGHD, isolated growth hormone deficiency; KS, Kallmann syndrome; LH, luteinizing hormone; TSH, thyroid-stimulating hormone

Adapted from Gregory LC, Dattani MT. J Clin Endocrinol Metab. Epub Nov 8, 2019

HESX1: A TRANSCRIPTIONAL REPRESSOR

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- Homeodomain transcriptional repressor expressed in early embryo (E6.5 in mouse) in region fated to form forebrain and pituitary
- Localised Rathke's pouch E9.5 days; no expression after E13.5
- Knock-out mice
  - Highly variable phenotype similar to septo-optic dysplasia in man
  - Anopthalmia/micropthalmia, pituitary dysgenesis, midline brain defects, olfactory bulb hypoplasia
- Human mutations associated with recessive/dominant SOD, CPHD and IGHD
  - Rare
  - Variably penetrant

E, embryonic day; CPHD, combined pituitary hormone deficiency; HESX1, HESX homeobox 1; IGHD, isolated growth hormone deficiency; SOD, septo-optic dysplasia

Dattani MT, et al. Nat Genet. 1998;19:125-33

MRI APPEARANCES IN SOD ASSOCIATED WITH *HESX1* MUTATIONS

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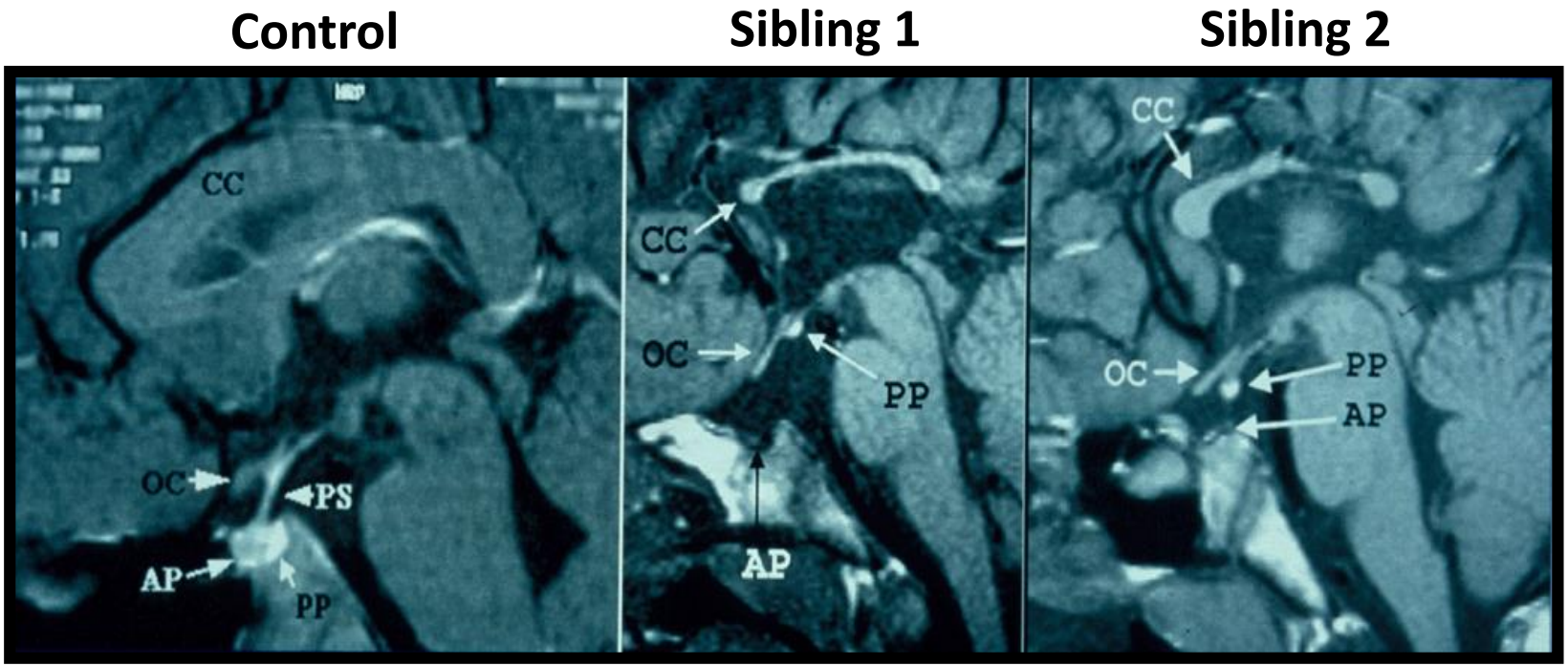
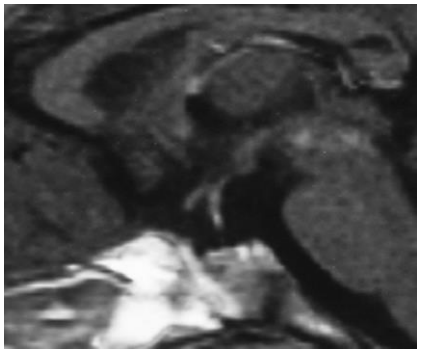


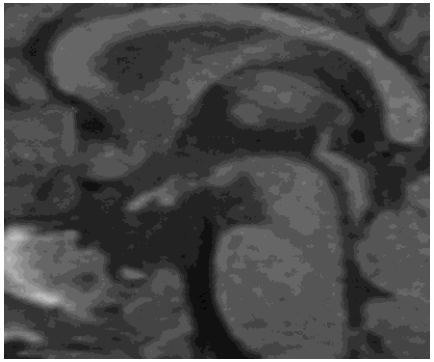
AP, anterior pituitary; CC, corpus callosum; HESX1, HESX homeobox 1; MRI, magnetic resonance imaging; OC, optic chiasm; PP, posterior pituitary; PS, pituitary stalk; SOD, septo-optic dysplasia

SOX3 MUTATIONS

- Located Xq26-27
- 446aa HMG transcription factor with 4 PA repeats
- Mutant mice: abnormal hypothalamus and pituitary
- Genetic duplications associated with hypopituitarism
- Loss of function mutations also associated with hypopituitarism



Sibling 1



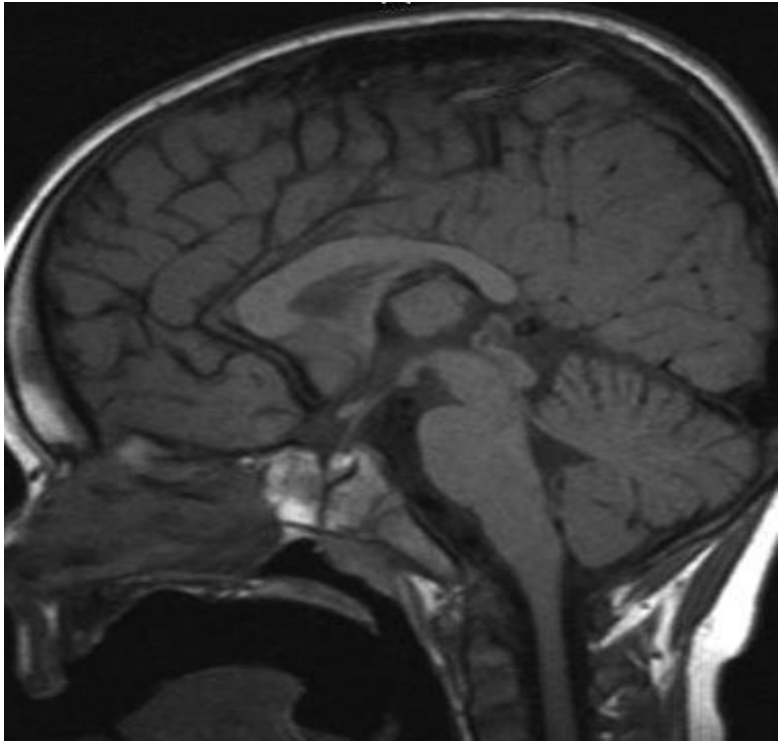
Sibling 2

aa, amino acid; HMG, high-mobility group; PA, polyalanine; SOX3, sex-determining region Y box 3

1. Kelberman D, et al. Endocr Rev. 2009;30:790-829. 2. Woods KS, et al. Am J Hum Genet. 2005;76:833-49

SOX3 DELETION

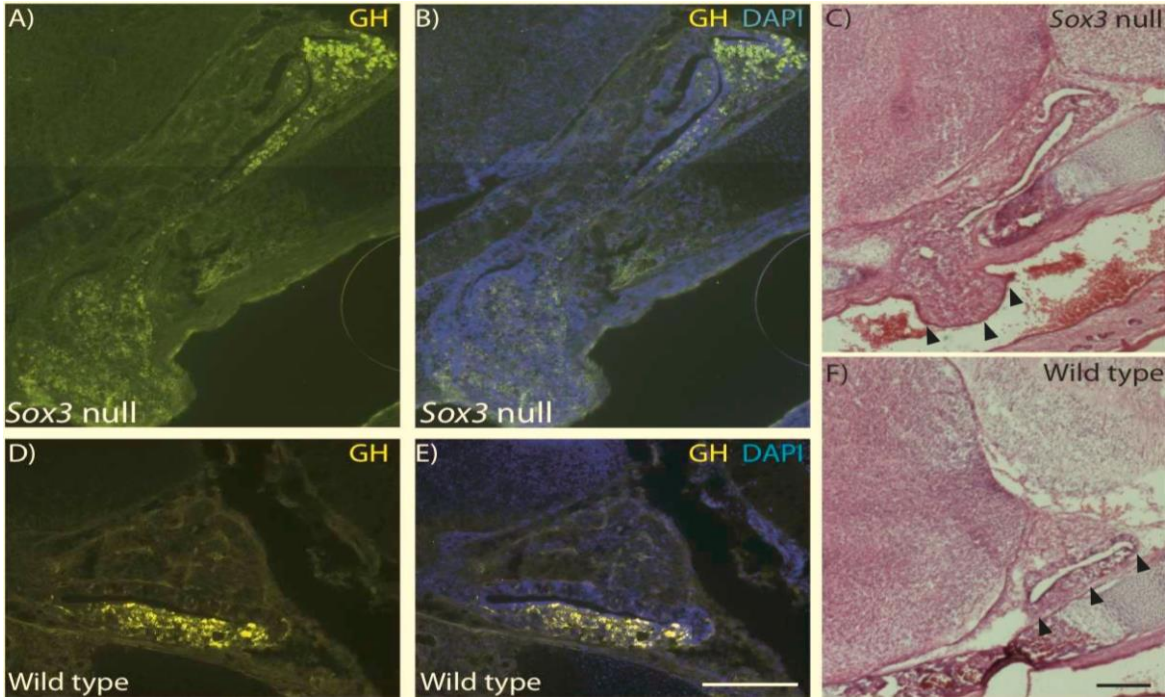
- Male patient with Haemophilia B, GH and gonadotrophin deficiencies; borderline low FT4
- De novo 2.31Mb deletion of Xq27.1-q27.2 incorporating *SOX3* and *F9* encoding Factor IX



FT4, free T4; GH, growth hormone; SOX3, sex-determining region Y box 3

Alatzoglou KS, et al. J Clin Endocrinol Metab. 2014;99:E2702-8

SOX3 NULL MICE SHOW PERSISTENT CRANIOPHARYNGEAL CANAL AT P1



SOX3, sex-determining region Y box 3

Alatzoglou KS, et al. J Clin Endocrinol Metab. 2014;99:E2702-8

OTX2 AND HYPOPITUITARISM

- Expressed early in murine development; important for forebrain/hypothalamic development – *HESX1* expression
- Mutations identified in patients with CPHD/IGHD:
  - Eye defects – retinal dystrophy, anophthalmia/microphthalmia
  - Cerebellar abnormalities
  - Variable hypopituitarism
  - APH, EPP (variable)

APH, anterior pituitary hypoplasia; CPHD, combined pituitary hormone deficiency; EPP, ectopic posterior pituitary; HESX1, HESX homeobox 1; IGHD, isolated growth hormone deficiency; OTX2, orthodenticle homeobox 2

LHX3/LHX4 MUTATIONS

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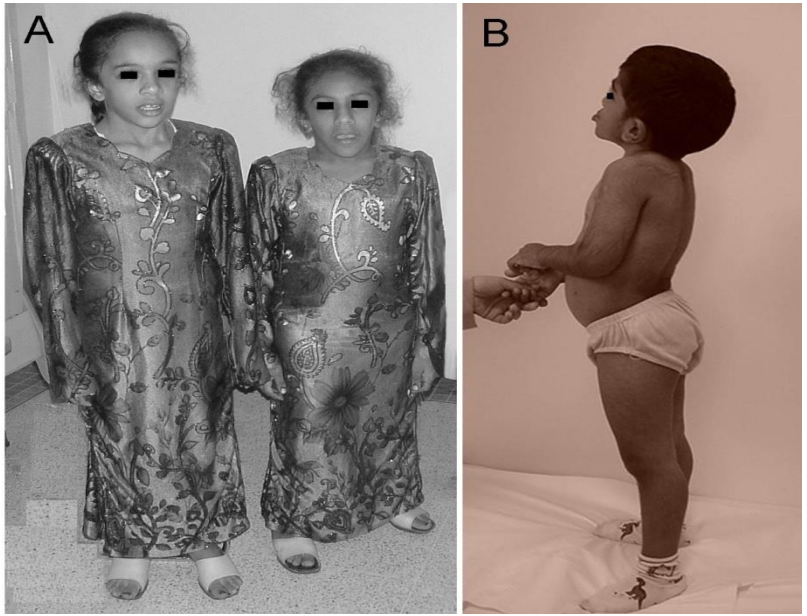
Gene affected	Inheritance	Phenotype
LHX3	**Recessive (deletion, missense)**	**GH, TSH, PRL, LH, FSH deficiencies** **APH** **Eutopic PP** **Variable short stiff neck** **Enlarged pituitary (n=2)**
LHX4	**Dominant (splice site)** **Recessive in mouse**	**GH, TSH, cortisol, Gn deficiencies** **APH** **Pituitary cysts** **EPP** **Abnormal cerebellar tonsils**

APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; FSH, follicle-stimulating hormone; GH, growth hormone; Gn, gonadotropin; LH, luteinizing hormone; PP, posterior pituitary; PRL, prolactin; LHX, LIM homeobox; TSH, thyroid-stimulating hormone

1. Netchine I, et al. Nat Genet. 2000;25:182-6. 2. Bhangoo AP, et al. J Clin Endocrinol Metab. 2006;91:747-53. 3. Machinis K, et al. Am J Hum Genet. 2001;69:961-8

NOVEL CH PHENOTYPE



- 3 siblings with panhypopituitarism
- Sensorineural hearing loss
- Skeletal defects
- Skin defects
- *LHX3* deletion – LHX3 expressed in pituitary, inner ear and spinal cord

CH, congenital hypopituitarism; LHX3, LIM homeobox 3

Rajab A, et al. Hum Mol Genet. 2008;17:2150-9

NOVEL INSIGHTS INTO *LHX4* MUTATIONS

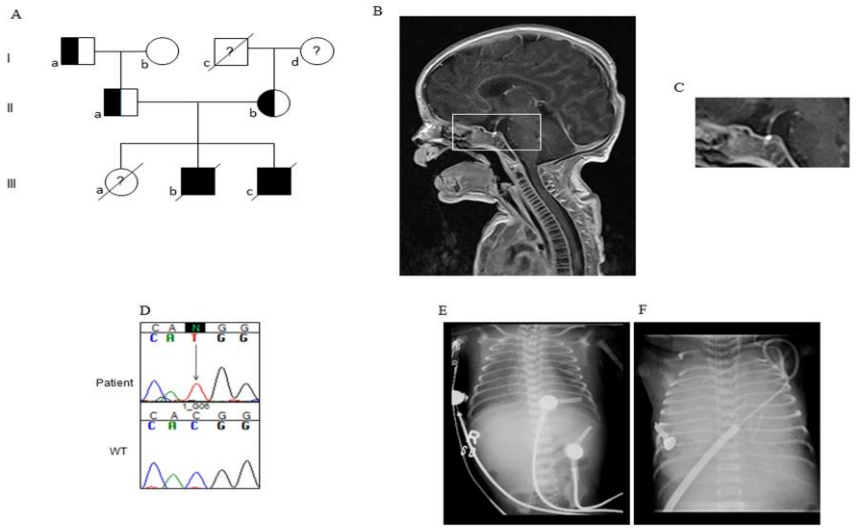
- In humans, heterozygous mutations in *LHX4* are associated with combined pituitary hormone deficiency
  - Mice with heterozygous mutations are normal
- Mice homozygous for *LHX4* mutations die shortly after birth with immature lungs that do not inflate
- *LHX4* null mice exhibit incomplete pituitary gland development

LHX4, LIM homeobox 4

1. Sheng HZ, et al Trends Genet. 1999;15:236-40. 2. Machinis K, et al. Am J Hum Genet. 2001;69:961-8

NOVEL INSIGHTS INTO *LHX4* MUTATIONS

- Two deceased male Pakistani patients born to non-consanguineous parents
- Homozygous *LHX4* c.377C>T, p.T126M located LIM2 domain, highly conserved
- Both had panhypopituitarism, SGA, mid-facial hypoplasia, microphallus with poorly developed scrotum
- Further daughter with a depressed nasal bridge and cleft palate (DNA not available)
- Rapid commencement of hydrocortisone and thyroxine in the 2 boys; all three children died within the first week of life



LHX4, LIM homeobox 4; LIM2, lens intrinsic membrane protein 2; SGA, small for gestational age

Gregory LC, et al. J Clin Endocrinol Metab. 2015;100:2158-64

SHH, GLI2, HOLOPROSENCEPHALY AND HYPOPITUITARISM

- *Gli2* mediates actions of Sonic Hedgehog
- Heterozygous variably penetrant mutations associated with holoprosencephaly, abnormal pituitary function and craniofacial defects
- Hypopituitarism without midline defects
- Post-axial polydactyly, single central incisor and partial agenesis of CC associated

CC, corpus callosum; SHH, sonic hedgehog

Fang Q, et al. Endocr Rev. 2016;37:636-75

NEXT GENERATION SEQUENCING

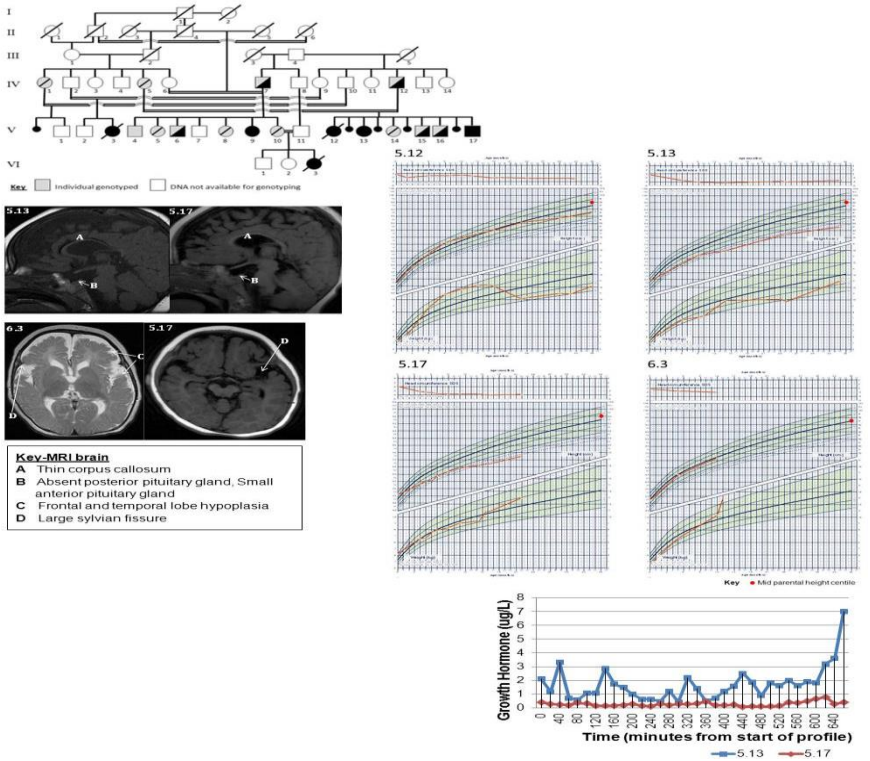
CONGENITAL HYPOPITUITARISM (CH): A NOVEL SYNDROME

- Middle-Eastern pedigree
- 6 children born to 3 pairs of first cousin parents
- Early onset DI, ACTH and TSH deficiencies
- Dysmorphic, blind
- Initial cerebral sparing with progressive microcephaly, intractable tonic/clonic seizures, cerebral palsy and global developmental delay
- Congenital dislocation of the hips
- Hydronephrosis, vesico-ureteric reflux and nephrogenic bladder present in all affected
- 3 died of sepsis

ACTH, adrenocorticotropic hormone; CH, congenital hypopituitarism; DI, diabetes insipidus; TSH, thyroid-stimulating hormone

Webb EA, et al. Brain. 2013;136(Pt 10):3096-105

MOLECULAR ANALYSIS



- Homozygosity mapping followed by exome sequencing
- Variant in *ARNT2* on chromosome 15 identified
  - Homozygous c.1373_1374dupTC
  - Recessive, parents and unaffected siblings heterozygous or homozygous for the normal allele
  - Frameshift with premature stop 52 amino acids later
  - Nonsense-mediated decay

ARNT2, aryl hydrocarbon receptor nuclear translocator 2

Webb EA, et al. Brain. 2013;136(Pt 10):3096-105

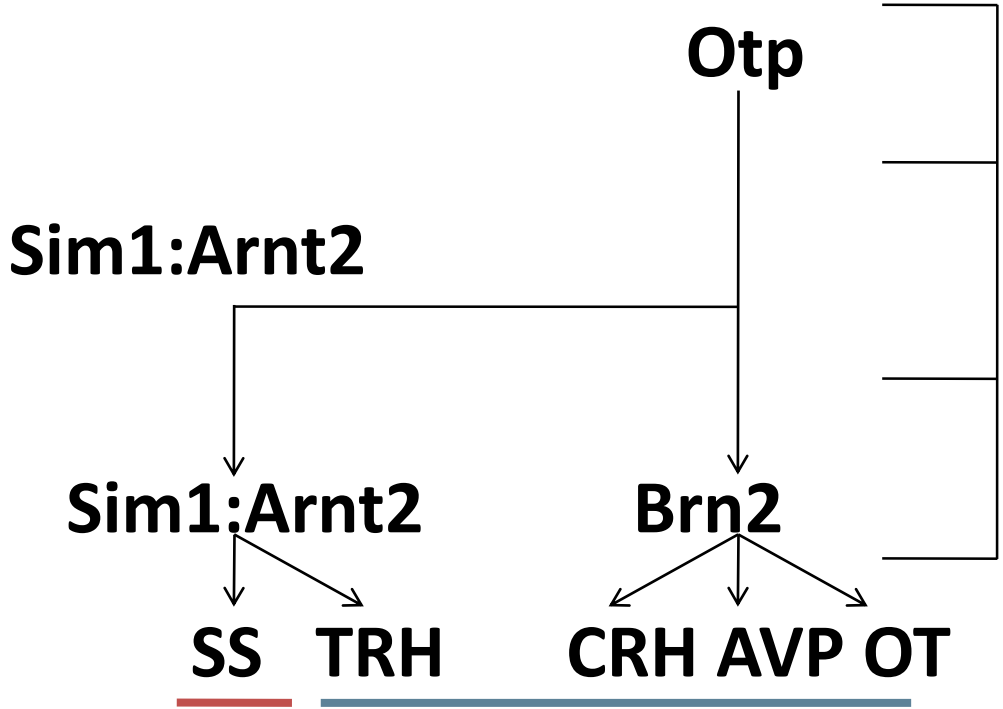
ARNT2

- Aryl hydrocarbon nuclear translocator 2
- Transcriptional regulator, member of the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) protein family – important for hypothalamic development
- Binds to DNA as a heterodimer with Aryl hydrocarbon receptor (AhR), Hypoxia-inducible factor 1α (Hif-1α) and Sim1
- Expressed widely
  - foetal brain including olfactory bulb, basal ganglia, rhinencephalon, midbrain, thalamus, hypothalamus
  - kidney, muscular layer of the urinary bladder, cochlea, inner layer retina
- Murine knockout:
  - Hypocellular/absent paraventricular, supraoptic and anterior periventricular nuclei
  - Absent/hypoplastic posterior pituitary glands, thin median eminence
  - Deficiencies in oxytocin, vasopressin, CRH, TRH and somatostatin
  - *SIM1* and *ARNT2* mutant mice die within the 1st week of life

AhR, aryl hydrocarbon receptor; ARNT2, aryl hydrocarbon receptor nuclear translocator 2; bHLH-PAS, basic helix-loop-helix-PER-ARNT-SIM; CRH, corticotropin-releasing hormone; Hif-1α, hypoxia-inducible factor 1α; SIM1, single-minded homolog 1; TRH, thyrotropin releasing hormone

Webb EA, et al. Brain. 2013;136(Pt 10):3096-105

HYPOTHALAMIC DEVELOPMENT



Cell proliferation and migration

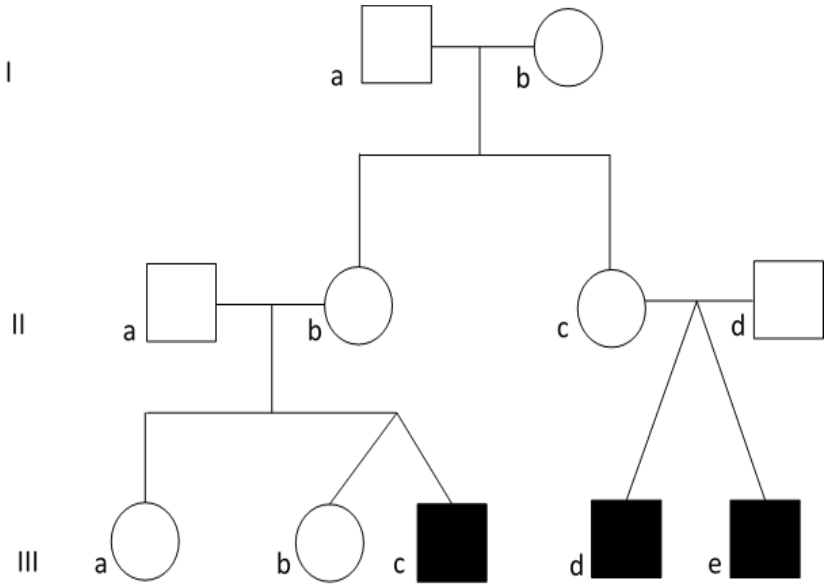
Cell differentiation

Discrete hypothalamic nuclei

AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; GH-RH, growth hormone-releasing hormone; OT, oxytocin; PVN, paraventricular nucleus; SON, supraoptic nucleus; SS, somatostatin; TRH, thyrotropin-releasing hormone

1. Webb EA, et al. Brain. 2013;136(Pt 10):3096-105. 2. Takahashi K, et al. Hypothalamus and Neurohypophysis. 2010. In: Lloyd R. (eds) Endocrine Pathology:. Springer, New York, NY

X-LINKED HYPOPITUITARISM WITH GLUCOSE DYSREGULATION



- Non-consanguineous Caucasian pedigree with three affected males
- Central hypothyroidism
- GHD
- Unique pancreatic phenotype: fluctuation between hyperinsulinaemic hypoglycaemia and hyperglycaemia
- Small anterior pituitary on MRI
- Micropenis
- Mild learning difficulties

GHD, growth-hormone deficiency; MRI, magnetic resonance imaging

Gregory LC, et al. EBioMedicine. 2019;42:470-80

ENDOCRINE PHENOTYPE

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Patient	Age at presentation years	Height at presentation cm (SDS)	Most recent height SDS (age in years)	HC SDS (age in years)	Peak GH to provocation ug/L	IGF1 ng/ml (NR)	IGFBP3 mg/L (NR)	Most recent cortisol nmol/L	FT4 (pre-treatment) pmol/L (NR)	TSH (pre-treatment) mU/L	Puberty
IIIc	1.13	58.8 (-6.7)	**–0.30 (8.8)**	-2.2 (7.5)	**<0.1 on profile**	<25	<0.5	315	**12.6 (12 - 22) Not treated**	5	N/A
IIId	2.2	71.5 (-4.4)	**–2.07 (14.1)**	-1.06 (13.1)	**1.1**	9 (20 - 158)	0.67 (1.2 - 3.7)	183	**11.4 (12 - 22)**	2.9	G4, TV 12, 25 mls
IIIe	2.2	69.5 (-5.2)	**–2.05 (14.1)**	-1.38 (13.1)	**0.7**	10 (20 - 158)	1.2 (1.2 - 3.7)	241	**11.3 (12 - 22)**	3.4	G4, TV 25, 20 mls

Mid-parental height -0.34 SDS

FT4, free T4; G, genitalia; GH, growth hormone; HC, head circumference; IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor-binding protein 3; NR, normal range; HCG, human chorionic gonadotrophin; SDS, standard deviation score; TSH, thyroid-stimulating hormone; TV, testicular volume

Gregory LC, et al. EBioMedicine. 2019;42:470-80

GLUCOSE DYSREGULATION

Patient	Glucose mmol/L (age in years)	Insulin mU/L (age in years)	Diazoxide treatment (age in years)	HbA1c in mmol/mol (NR 20-40)	Peak 2 hr glucose on OGTT in mmol/L (age in years)	Peak 2 hr insulin (mU/L)
IIIc	3.3 (0.25)	5.9 (0.25)	0.75 - 6.8		4.3 (8.9); BG 2.9 at 3 hours	8.6 at 3 hours when hypoglycaemic
IIId	3.4 (2.2)	6.8 (2.2)	2.5 - 6.7	47	13 (13.6) 2.7 5 hours post-glucose load	33.2 10.9 at time of hypoglycaemia
IIIe	3.2 (2.2)	4.9 (2.2)	2.5 - 6.7	43	13.5 (13.6) 2.9 (fasting)	30.5 2.5 (fasting at time of hypoglycaemia)

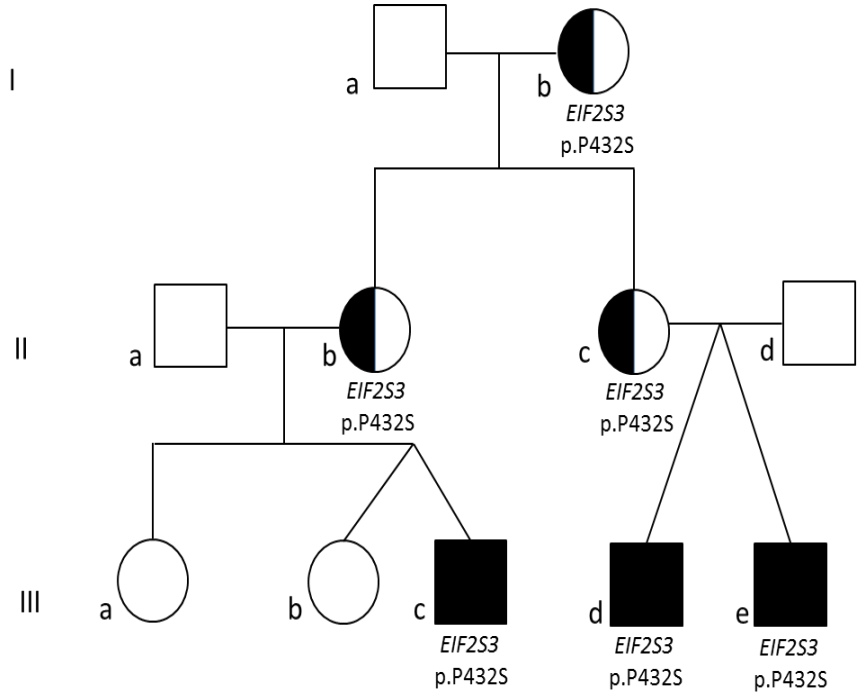
BG, blood glucose; HbA1c, haemoglobin A1c; NR, normal range; OGTT, oral glucose tolerance test

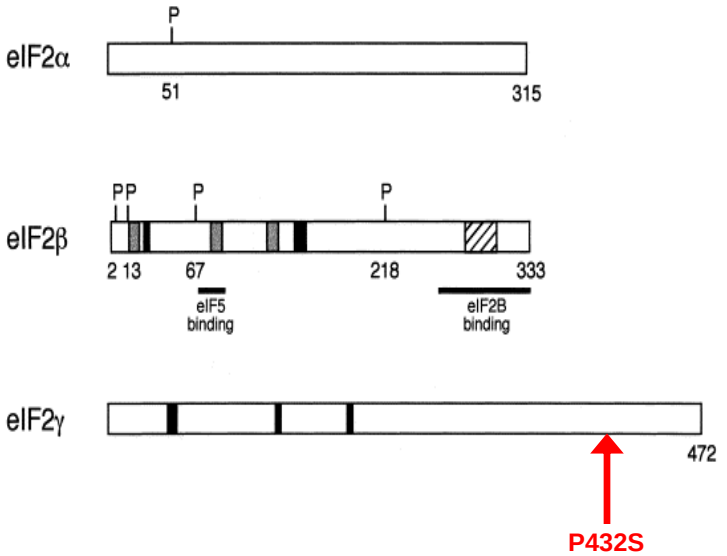
Gregory LC, et al. EBioMedicine. 2019;42:470-80

MOLECULAR DIAGNOSIS

Exome sequencing of the X chromosome revealed a novel variant in the *EIF2S3* gene

EIF2S3: ChrX_24091319 C/T, ***c.1294C>T,* p.P432S**



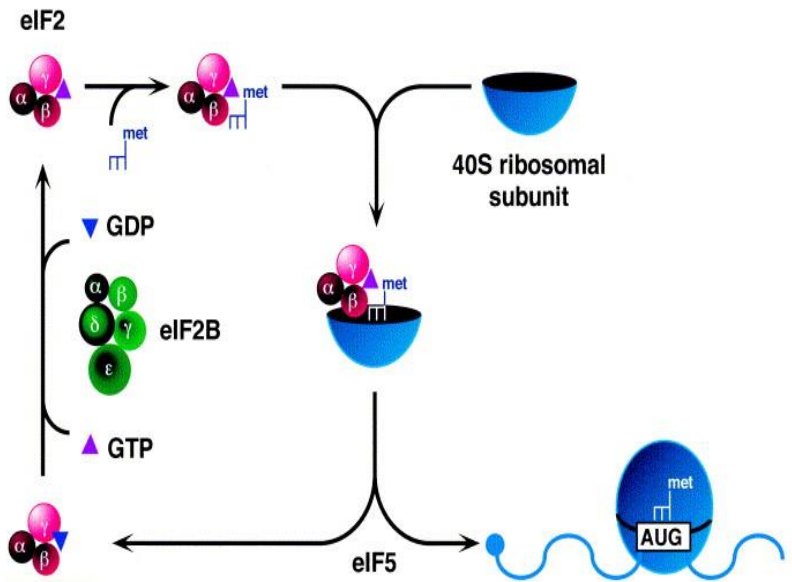


EIF, eukaryotic translation initiation factor; EIF2S3, eukaryotic translation initiation factor 2 subunit 3

1. Kimball SR, et al. Int J Biochem Cell Biol. 1999;31:25-9. 2. Gregory LC, et al. EBioMedicine. 2019;42:470-80

EIF2S3

- The *EIF2S3* gene encodes the gamma subunit of Eukaryotic translation initiation factor 2 (eIF2)
- eIF2γ is the largest subunit (52kDa) of this heterotrimeric GTP-binding protein and contains all 3 consensus GTP-binding domains
- eIF2 localises to the nucleus and functions in the early steps of protein synthesis by forming a ternary complex with GTP and Met-tRNAi
- Binds to the mRNA at the 5’ end to form a 43S pre-initiation complex, which scans mRNA to select the AUG start codon for protein synthesis



Taken from Kimball SR, et al. 1999

EIF, eukaryotic translation initiation factor; EIF2S3, eukaryotic translation initiation factor 2 subunit 3; GDP, guanosine diphosphate; GTP, guanosine triphosphate; mRNA, messenger RNA; met-tRNAi, methionyl initiator tRNA

1. Kimball SR, et al. Int J Biochem Cell Biol. 1999;31:25-9. 2. Gregory LC, et al. EBioMedicine. 2019;42:470-80

PREVIOUS STUDIES: MEHMO SYNDROME

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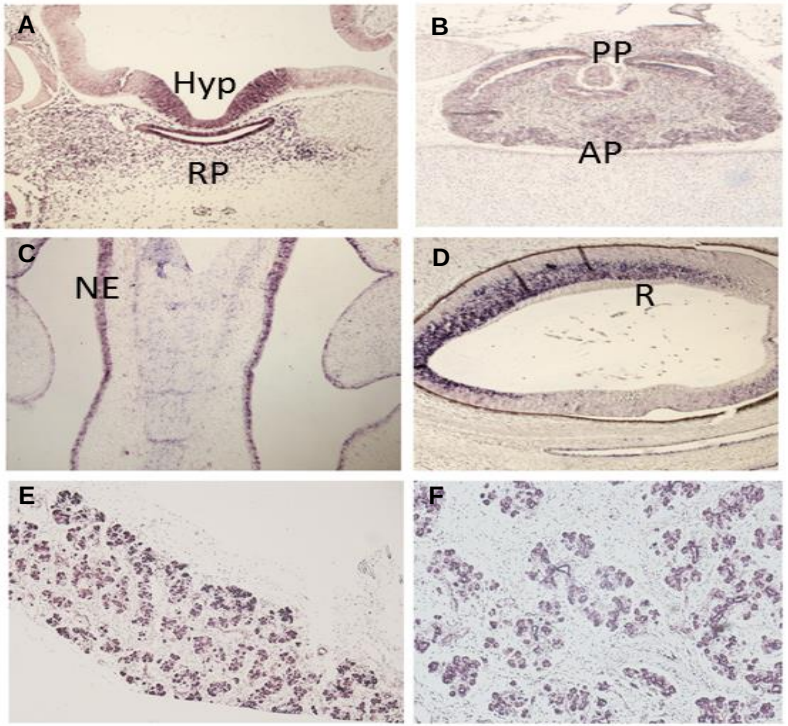
MEHMO: Mental retardation, Epilepsy, Hypogonadism, Microcephaly, Obesity

Borck et al. 2012 Mol Cell	Moortgat et al. 2016 AJMG	Skopkova et al. 2017 Hum Mutat Stanik J et al. 2018 Physiol Res	This study – Pedigree 1
EIF2S3, p.I222T in the highly conserved GTP-binding (G) domain	EIF2S3, p.I259M and p.I465Sfs*4 in two unrelated pedigrees in the C-terminal domain	EIF2S3, p.I465Sfs*4 and pS108R in four unrelated pedigrees	EIF2S3, p.P432S in the C-terminal domain
Three males: 2 brothers and maternal uncle • Intellectual disability (moderate to severe) • Microcephaly • Short stature with GHD in two patients • Facial dysmorphic features • Epilepsy • Thin corpus callosum on MRI • Enlarged lateral ventricles on MRI • Obesity	Three males: 2 brothers, 1 unrelated male • Severe intellectual disability • Microcephaly • GHD • Hypoglycaemia • Epilepsy • Thin corpus callosum on MRI • Normal pituitary and stalk on MRI • Global white matter loss on MRI	Four unrelated male patients: • Microcephaly • Seizures • Hypotonia (axial) • Hypertonia (peripheral) • Hypogonadism • Developmental delay • Obesity (Infancy onset) • Neonatal hypoglycaemia • Early onset diabetes	Three males: 2 brothers and maternal male cousin • Central hypothyroidism • GHD • Unique pancreatic phenotype: fluctuation between hyperinsulinaemic hypoglycaemia and hyperglycaemia • Small anterior pituitary on MRI • Thin corpus callosum on MRI

GHD, growth hormone deficiency; GTP, guanosine triphosphate; MRI, magnetic resonance imaging

Gregory LC, et al. EBioMedicine. 2019;42:470-80

EXPRESSION PATTERN OF *EIF2S3*

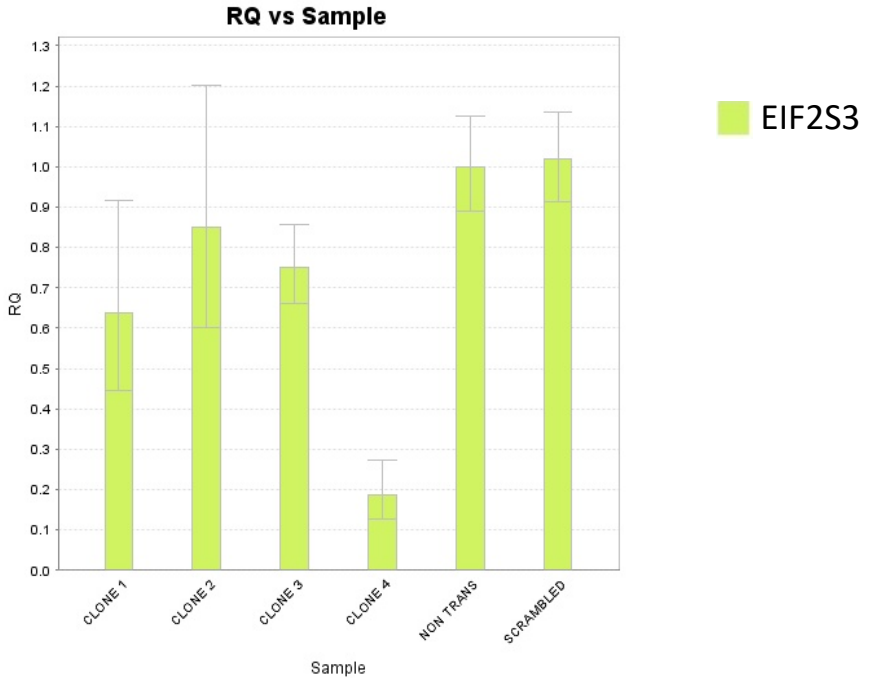


AP, anterior pituitary; EIF2S3, eukaryotic translation initiation factor 2 subunit 3; Hyp, hypothalamus; NE, nasal epithelium; PP, posterior pituitary; RP, Rathke's pouch; R, retina

Gregory LC, et al. EBioMedicine. 2019;42:470-80

KNOCKDOWN OF EIF2S3 IN A PANCREATIC CELL LINE

Stable knockdown of EIF2S3 in pancreatic cell line 1.1B4, a hybrid cell line formed by the electrofusion of a primary culture of human pancreatic islets with PANC-1, a human pancreatic ductal carcinoma cell line

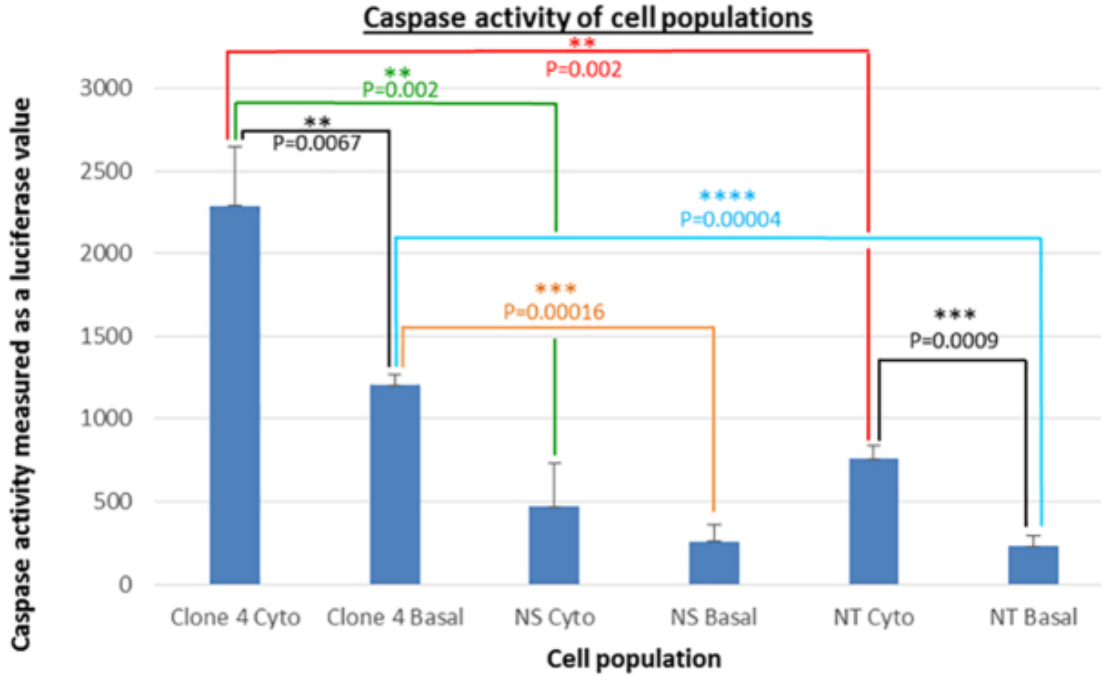


Relative quantification of *EIF2S3* expression, against *GAPDH*, *β-ACTIN* and *HPRT* in cDNA derived from transduced 1.1B4 cells, compared to non-transduced cells

cDNA, complementary DNA; EIF2S3, eukaryotic translation initiation factor 2 subunit 3; GAPGH, glyceraldehyde 3-phosphate dehydrogenase; HPRT, hypoxanthine phosphoribosyltransferase; RQ, relative quantification

Gregory LC, et al. EBioMedicine. 2019;42:470-80

INCREASED APOPTOSIS IN EIF2S3 KNOCK DOWN PANCREATIC CELLS



Significant increase in caspase activity in the *EIF2S3* KO cell line compared to controls

Cyto, cytokine; EIF2S3, eukaryotic translation initiation factor 2 subunit 3; KO, knockout; NS, scrambled non-silencing; NT, non-transduced

Gregory LC, et al. EBioMedicine. 2019;42:470-80

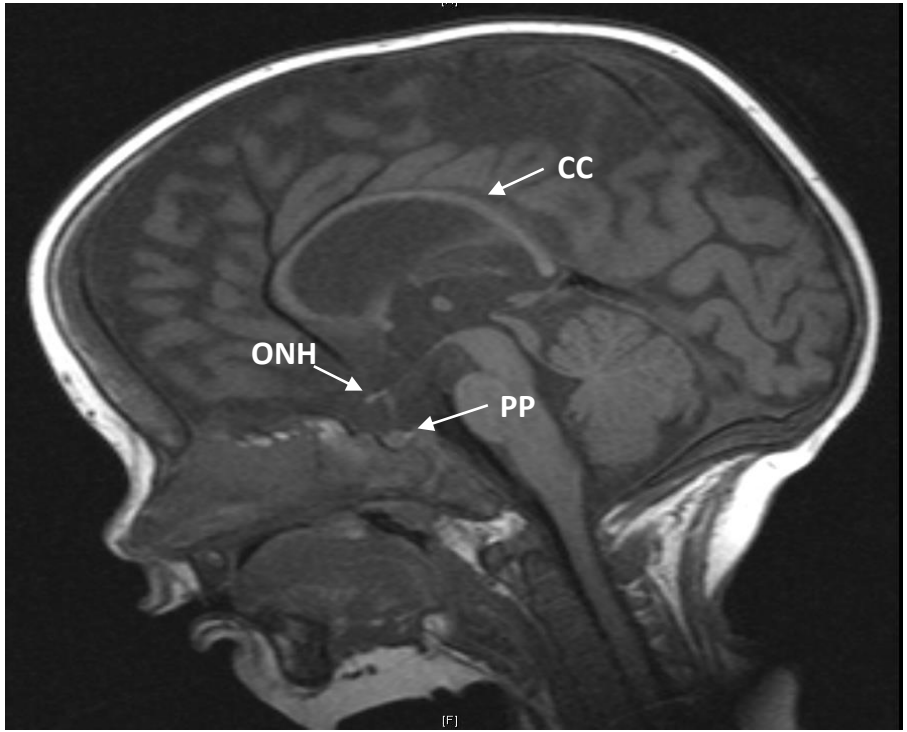
MAGEL2 C.1996DUPC, P.Q666PFS*47

Patient	Clinical phenotype
1 - female (GOSH, London)	• GHD • Arthrogryposis • Dysmorphic features: Bulbar palsy • Developmental delay • Visual concerns - Squint, mild optic nerve hypoplasia (ONH), cerebral visual impairment • Central sleep apnoea • Scoliosis • Gastro-oesophageal reflux
2-3 - female non- (GOSH, London)	*De novo MAGEL2* c.1996dupC, p.Q666Pfs*47 • Global developmental delay • ONH • Central sleep apnoea • Scoliosis
4 - Male (Santiago hospital, Chile)	• MPHD: GHD, ACTH insufficiency • Hyperprolactinaemia • Arthrogryposis • Dysmorphic features • Micrognathia • Central sleep apnoea • Cryptorchidism with bilateral orchidopexies • Strabismus • MRI: Hypoplastic pituitary

ACTH, adrenocorticotropic hormone; GHD, growth hormone deficiency; GOSH, Great Ormond Street Hospital; MPHD, multiple pituitary hormone deficiencies; MRI, magnetic resonance imaging; ONH, optic nerve hypoplasia

Gregory LC, et al. J Clin Endocrinol Metab. 2019;104:5737-50

MRI OF TWIN WITH *MAGEL2* C.1996DUPC, P.Q666PFS*47

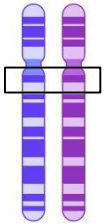


CC, corpus callosum; MRI, magnetic resonance imaging; ONH, optic nerve hypoplasia; PP, posterior pituitary

Gregory LC, et al. J Clin Endocrinol Metab. 2019;104:5737-50

MAGEL2

PWS
15q11-q13



5 maternally imprinted (paternally expressed) genes:
*MKRN3, **MAGEL**2, NDN, NPAP1, SNURF-SNRPN*

Variable features reminiscent of PWS but with autism spectrum disorder (ASD) and contractures of the small finger joints (arthrogryposis) without hyperphagia and subsequent obesity *(Schaaf 2013)*

Schaaf-Yang syndrome (SHFYNG) *(Schaaf et al 2013)*

PWS, Prader-Willi Syndrome

Schaaf CP, et al. Nat Genet. 2013;45:1405-8

MAGEL2 BACKGROUND

- *MAGEL2* is a member of the type II MAGE gene family involved in neurogenesis and brain function[1,2]
- The role of *MAGEL2*:
  - Enhance ubiquitin ligase activity[3]
  - Act as a regulator of retrograde transport
  - Promote endosomal F-actin assembly
  - Involved in the regulation of the circadian clock[4]
- *Magel2*-null mice present with similar features to PWS in humans: neonatal growth retardation, excessive weight gain after weaning, impaired hypothalamic regulation and reduced fertility[5-8]
- POMC neuron activity and its communication with downstream targets is significantly compromised[9]
- Oxytocin neuron activity is suppressed[10]

POMC, proopiomelanocortin; PWS, Prader-Willi Syndrome

1. Bischof JM, et al. Dev Dyn. 2003;228:475-9. 2. López-Sánchez N, et al. Physiol Genomics. 2007;30:156-71. 3. Doyle JM, et al. Mol Cell. 2010;39:963-74. 4. Tacer KF, et al. Biochem J. 2017;474:2177-90. 5. Bischof JM, et al. Hum Mol Genet. 2007;16:2713-9. 6. Hao YH, et al. Mol Cell. 2015;59:956-69. 7. Mercer RE, et al. Am J Med Genet B Neuropsychiatr Genet. 2009;150B:1085-99. 8. Tenesse AA, et al. Endocrinol 2011;152:967-78. 9. Oncul M, et al. Hum Mol Genet. 2018;27:3129-36. 10. Ates T, et al. Neurobiol Dis. 2019;121:58-64

MAGEL2 MUTATIONS

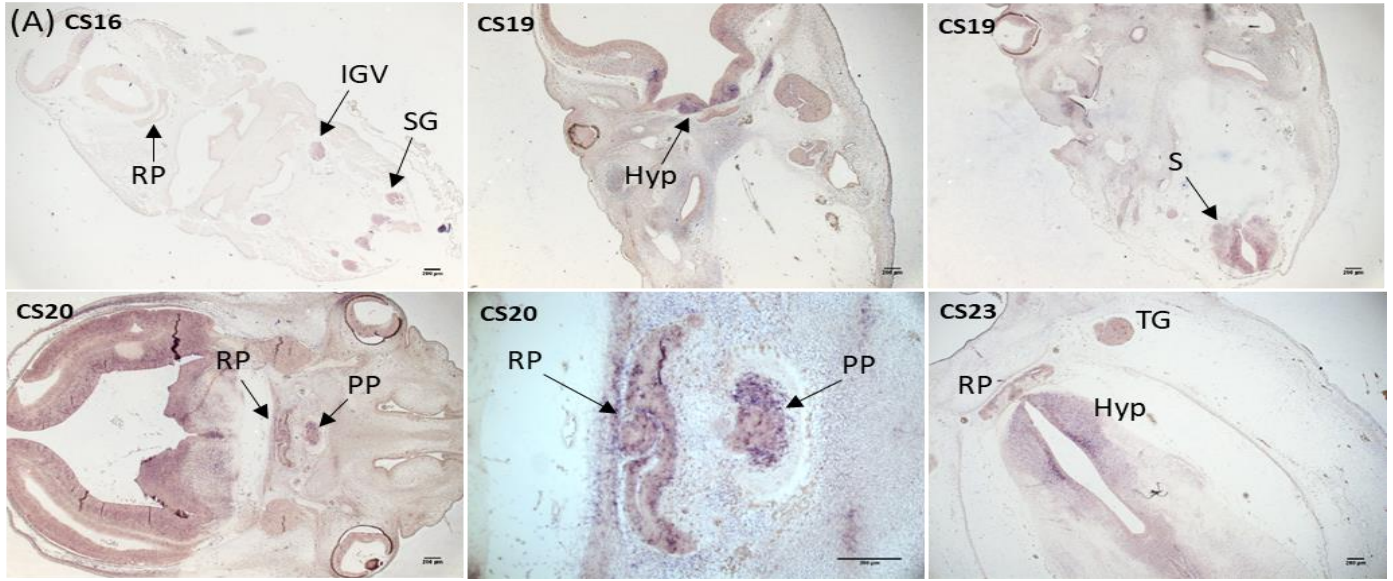
- The *MAGEL2* mutation has been identified in multiple SHFYNG patients:
  - c.1996**dup**C, p.Q666Pfs*47
  - c.1996**del**C, p.Q666fs
- The majority had arthrogryposis (ranging in severity), short stature and hypogonadism – all common features seen in SHFYNG patients
- ***Enya et al*** - Two siblings and an unrelated female patient with SHFYNG carried MAGEL2 truncations, p.Q638* and p.S1044* respectively[1]:
  - Central **diabetes insipidus** and **gonadotrophin deficiency** (1 sibling)
  - **Panhypopituitarism** including **GHD, central hypothyroidism, adrenal insufficiency, gonadotrophin deficiency**, with a hypoplastic pituitary gland in female patient
- ***Jobling et al*** – *MAGEL2* mutations give rise to Chitayat-Hall syndrome. Chitayat-Hall syndrome and SHFYNG share a common aetiology[2]

GHD, growth hormone deficiency; SHFYNG, Schaaf-Yang syndrome

1. Enya T, et al. Am J Med Genet A. 2018;176:707-11. 2. Jobling R, et al. J Med Genet. 2018;55:316-21

HUMAN EXPRESSION OF *MAGEL2* DURING EMBRYONIC BRAIN DEVELOPMENT

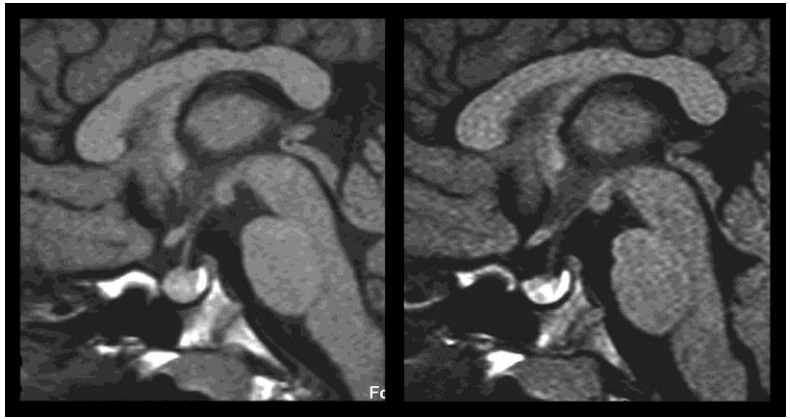
CS16 (GA: 5.5 weeks), CS19 (GA: 6 weeks), CS20 (GA: 7 weeks), CS23 (GA: 8 weeks)



CS, Carnegie stage; GA, gestational age; Hyp, hypothalamus; IGV, inferior ganglion of vagus nerve; PP, posterior pituitary; RP, Rathke's pouch; S, spinal cord; SG, spinal ganglia; TG, trigeminal ganglia

Gregory LC, et al. J Clin Endocrinol Metab. 2019;104:5737-50

PROP1 DEFICIENCY

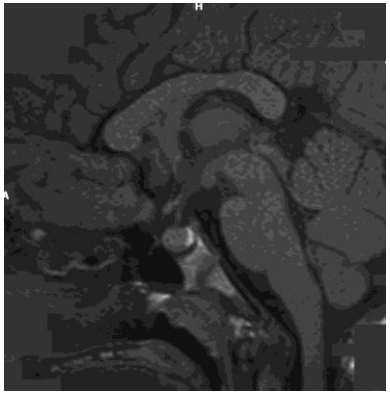


JUNE 2002 NOV 2002

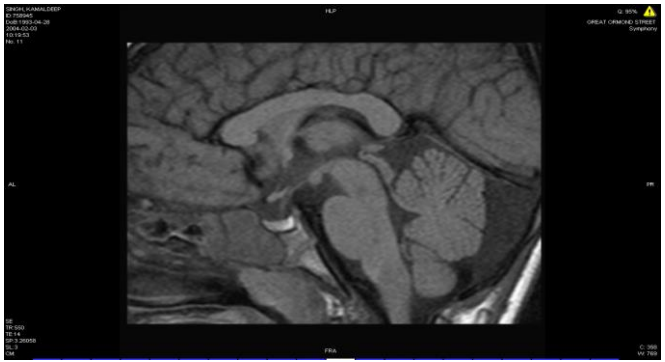
GH, Prolactin, TSH and LH/FSH deficiencies

Autosomal recessive

Phenotypic variability



JUNE 2003



FEBRUARY 2004

FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone. Fang Q, et al. Endocr Rev. 2016;37:636-75

SEVERE DWARFISM IN A BOY WITH POU1F1 DEFICIENCY

- Pituitary-specific transcription factor
- Determination, proliferation and survival of thyrotrophs, lactotrophs and somatotrophs
- Expression of *GH, PRL, βTSH, GHRHR* genes
- GH, PRL and variable TSH deficiency
- Autosomal recessive/dominant

GH, growth hormone; GHRHR, growth-hormone-releasing hormone receptor; PRL, prolactin; TSH, thyroid-stimulating hormone

Turton JP, et al. Clin Endocrinol (Oxf). 2012;76:387-93

GENES CURRENTLY IMPLICATED IN CONGENITAL HYPOPITUITARISM

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Gene with reported variants	Phenotype	Mode of inheritance
ARNT2	CPHD, congenital abnormalities of the kidneys and urinary tract	Recessive
CDON	PSIS	Dominant
EIF2S3	GHD, TSHD, Glucose dysregulation, MEHMO syndrome	X-linked
FGF8	HH/KS; HPE	Dominant
FGFR1	HH/KS, SOD	Dominant
FOXA2	CPHD, HI, childhood-onset diabetes, choroidal coloboma, biliary atresia (cardiac/endoderm-derived organ abnormalities)	Dominant
GH1	IGHD Type IA	Recessive
	IGHD Type IB	Recessive
	IGHD Type II	Dominant
GHRHR	IGHD Type IB	Recessive or Dominant (rare)
GLI2	HPE, IGHD/CPHD, polydactyly, single central incisor	Dominant: haploinsufficiency
GPR161	PSIS	Recessive
HESX1	IGHD, CPHD, SOD	Dominant or Recessive
IFT172	GHD, retinopathy, metaphyseal dysplasia, renal failure (ciliopathies)	Compound heterozygous
IGSF1	TSHD, hyperprolactinaemia, transient GHD; usually with macro-orchidism	X-linked
KAL1	HH/KS	X-linked
KCNQ1	GHD, maternally inherited gingival fibromatosis	Dominant
LHX3	CPHD, short neck with limited rotation	Recessive

CPHD, combined pituitary hormone deficiency; GHD, growth hormone deficiency; HH, hypogonadotropic hypogonadism; HI, congenital hyperinsulinism; HPE, holoprosencephaly; IAD, isolated adrenocortical deficiency; IGHD, isolated growth hormone deficiency; KS, Kallmann syndrome; MEHMO, mental retardation, epileptic seizures, hypogonadism with hypogenitalism, microcephaly and obesity; PSIS, pituitary stalk interruption syndrome; SOD, septo-optic dysplasia; TSHD, thyroid-stimulating hormone deficiency.
Adapted from Gregory LC, Dattani MT. [Published online ahead of print Nov 8, 2019]. J Clin Endocrinol Metab. doi: 10.1210/clinem/dgz184

GENES CURRENTLY IMPLICATED IN CONGENITAL HYPOPITUITARISM

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Gene with reported variants	Phenotype	Mode of inheritance
LHX4	CPHD, Chiari malformation, cerebellar abnormalities, respiratory distress	Dominant or Recessive
OTX2	IGHD, CPHD, SOD, anophthalmia/microphthalmia, retinal dystrophy	Dominant: haploinsufficiency or dominant negative
PCSK1	IAD, GHD, TSHD, DI, malabsorption	Dominant, Compound heterozygous
PNPLA6	Oliver–McFarlane and Laurence–Moon syndrome; GH and gonadotrophin deficiencies	Recessive
POMC	IAD; early-onset obesity and red hair pigmentation	Recessive
POU1F1	GH, TSH and ACTH deficiencies	Dominant or Recessive
PROKR2	HH/KS	Recessive
PROP1	CPHD, pituitary tumors	Recessive
RAX	Anophthalmia/microphthalmia, CPHD, DI, and Cleft Palate	Recessive or Compound heterozygous

Gene with reported variants	Phenotype	Mode of inheritance
RAX	Anophthalmia / microphthalmia, CPHD, DI, and Cleft Palate	Recessive or Compound heterozygous
RNPC3	IGHD	Recessive
ROBO1	PSIS	Dominant
SOX2	HH, anophthalmia/ microphthalmia, learning difficulties, hypothalamo-pituitary tumours	Dominant
SOX3	GHD, CPHD, absent infundibulum, persistent craniopharyngeal canal	X-linked
TBL1X	TSHD, ASD	X-linked
TBX19	IAD	Recessive
TCF7L1	SOD	Dominant
TRHR	TSHD	Recessive
TSHB	TSHD	Recessive

ACTH, adrenocorticotropic hormone; ASD, autism spectrum disorder; CPHD, combined pituitary hormone deficiency; DI, diabetes insipidus; GH, growth hormone; GHD, growth hormone deficiency; HH, hypogonadotropic hypogonadism; IAD, isolated adrenocortical deficiency; IGHD, isolated growth hormone deficiency; KS, Kallmann syndrome; PSIS, pituitary stalk interruption syndrome; SOD, septo-optic dysplasia; TSH, thyroid-stimulating hormone; TSHD, thyroid-stimulating hormone deficiency.
Adapted from Gregory LC, Dattani MT. [Published online ahead of print Nov 8, 2019]. J Clin Endocrinol Metab. doi: 10.1210/clinem/dgz184

NEW GENES?

- *TCF7L1* – SOD
- *KCNQ1* – CPHD, maternally inherited gingival fibromatosis
- *FOXA2* – Congenital Hyperinsulinism, MPHD, craniofacial dysmorphism, coloboma
- *RAX* – Anophthalmia, CLAP, MPHD

- ***IFT172*: ciliopathy; APH, EPP, retinopathy, metaphyseal dysplasia, renal failure**
- ***GPR161*: Pituitary stalk interruption syndrome**
- ***CDON*: PSIS – Sonic Hedgehog signaling protein**
- ***ROBO1*: PSIS – Slit/Robo signalling mediates axonal guidance**

APH, anterior pituitary hypoplasia; CLAP, cleft lip, alveolus and palate; CPHD, combined pituitary hormone deficiency; EPP, ectopic posterior pituitary; MPHD, multiple pituitary hormone deficiencies; PSIS, pituitary stalk interruption syndrome; SOD, septo-optic dysplasia

1. Adapted from Gregory LC, Dattani MT. J Clin Endocrinol Metab. Epub Nov 8, 2019. 2. Fang Q, et al. Endocr Rev. 2016;37:636-75

CONCLUSIONS

- Pleiotropic phenotypes – hypothalamic v pituitary
- Mutations/variations in genes implicated in pituitary development rare
- Variable inheritance
- Variably penetrant
  - Role of other genes
  - Role of environmental factors
- Evolution of phenotypes eg *PROP1, GH1*
- Careful interpretation required of any changes
- Novel causative pathways emerging eg 100K Genome project
- Care with genetic counseling

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Collaborators

Alejandro Martinez-Aguayo
Angham Mutair
Khadija Humayun
Sally Camper
Paul Letissier
Alison Salt
Naomi Dale
Simon Rhodes
Maria Arriazu
Referring clinicians

GOSGENE

PATIENTS AND FAMILIES

UCL GOS ICH

Juan-Pedro Martinez-Barbera
Carles Gaston-Massuet
Louise Gregory
Dan Kelberman
Sandy Alatzoglou
Emma Webb
Cynthia Andoniadou
Kathryn Woods
Mark McCabe
Dave McNay
James Turton
Luciani Carvalho
Maria Bitner-Glindzicz
Su Jayakody
Dianne Gerrelli
Jane Sowden
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