Appendix - *IGSF10* regulates embryonic GnRH neuronal migration and mutations result in delayed puberty

Howard SR, Guasti L, Ruiz-Babot G, Mancini A, David A, Storr HL, Metherell LA, Sternberg MJE, Cabrera CP, Warren HR, Barnes MR, Quinton R, de Roux N, Young J, Guiochon-Mantel A, Wehkalampi K, André V, Gothilf Y, Cariboni A, Dunkel L

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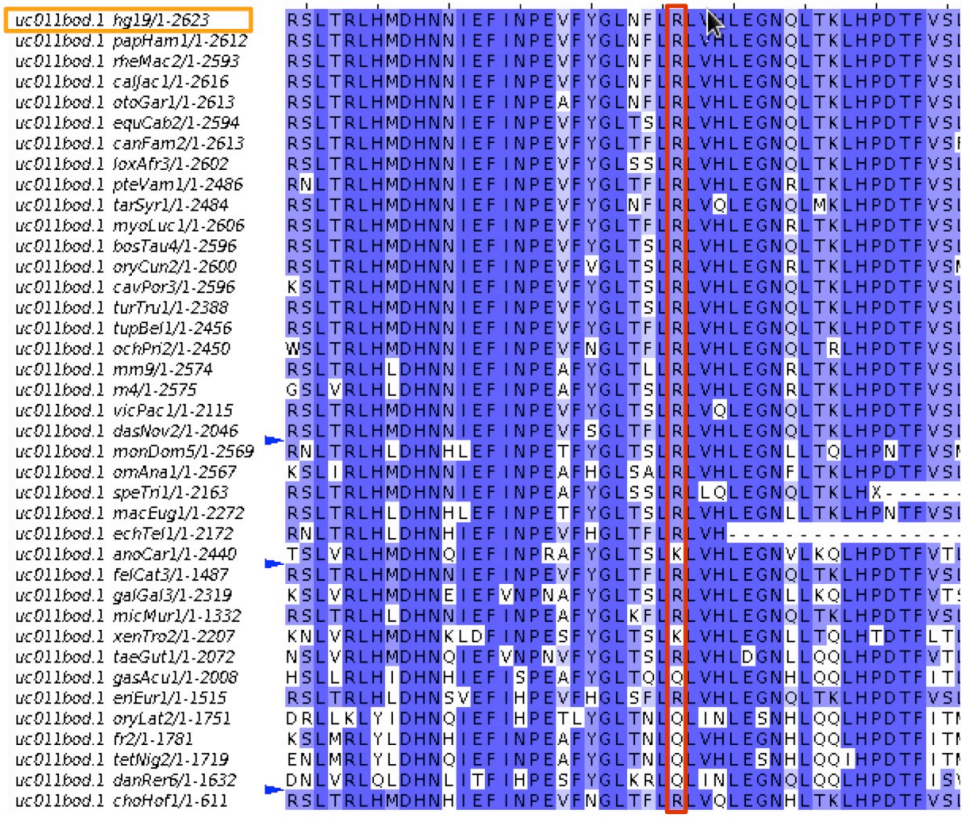
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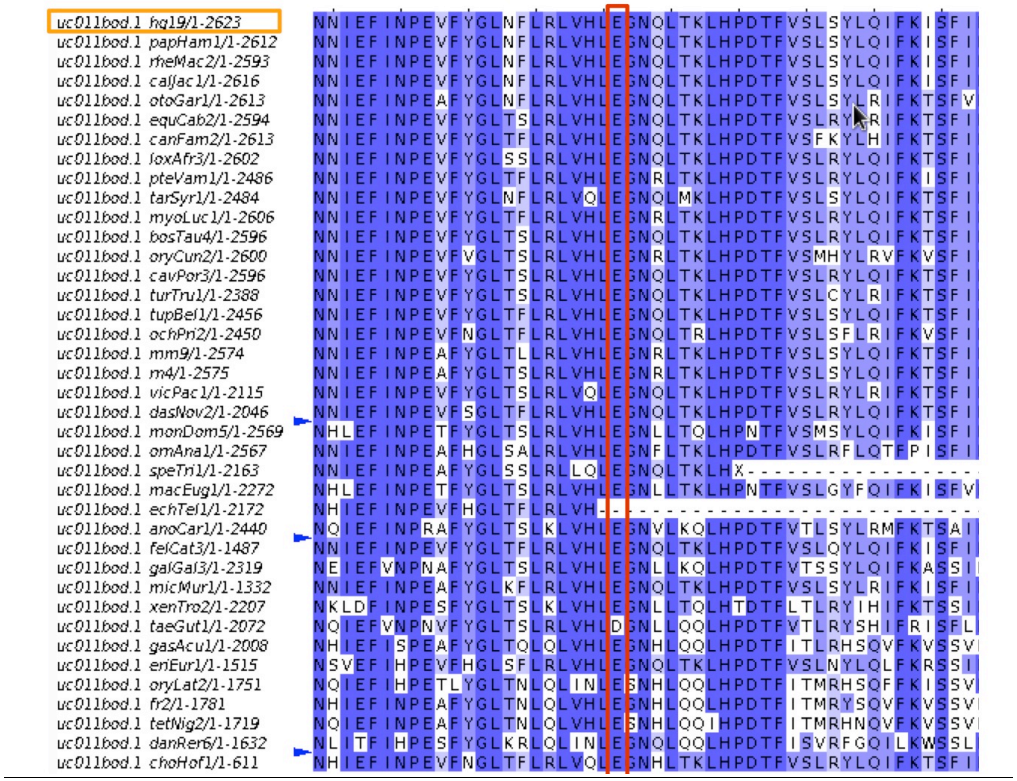
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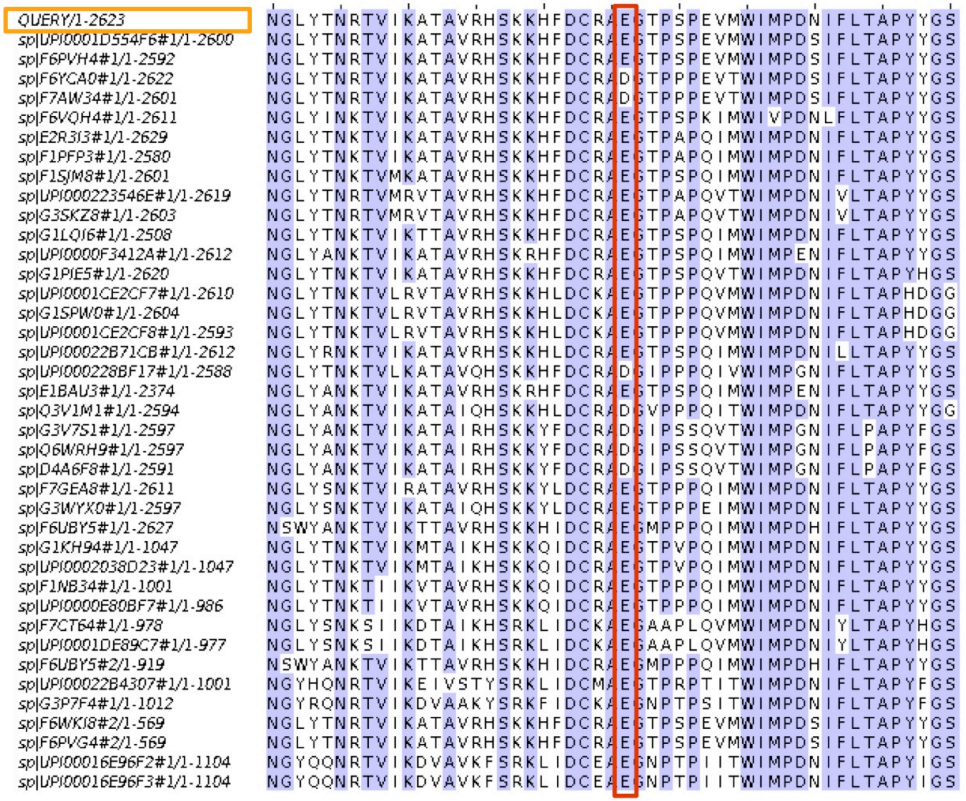
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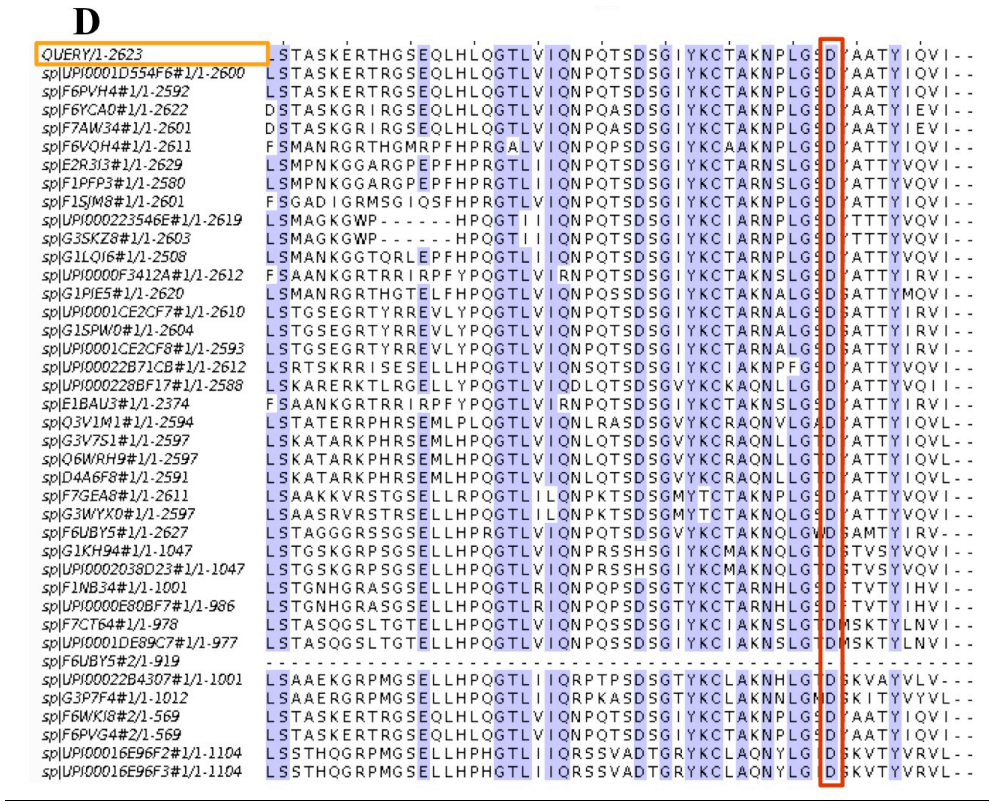
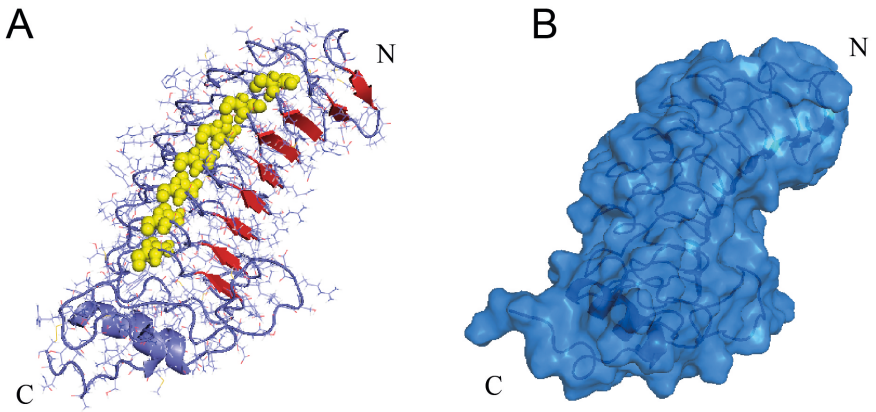


Appendix Figure S1

Multiple sequence alignment (m.s.a.) for all four residues harboring *IGSF10* mutations.

M.s.a. for residues R156 (A), E161 (B), E2264 (C) and D2614 (D). M.sa. was generated using Polyphen2 version 2.2.2, which retrieves homologues (both orthologs and paralogs, here displayed according to their Uniprot Id) using Blast+. M.s.a. is dislayed using Jalview applet. Residues are colored according to the percentage of the residues in each column that agree with the consensus sequence (percentage identity color scheme in Jalview, dark blue >80%, white < 40%).



Appendix Figure S2

Tertiary structure of LRR Region I.

Panel A: Beta strands are displayed in red and the asparagine ladder in yellow. Panel B: the ‘arc’ shape of LRR region I.

Appendix Figure S3
Efficacy of Sp-MO.
Nucleotide sequence obtained from RNA of *Igsf10* Sp-MO injected embryos showing an insertion of 97 nucleotides in the splice site of the second exon. Apostrophes denote homology.

Appendix Table S1: Rare Variant Burden Testing post Targeted Exome Sequencing

Gene	Total number of rare (MAF <2.5%) and predicted damaging* variant alleles in probands and in the reference population		P value	Adjusted P value**
	In DP cohort (n=49)	In ExAC Finn database (n=3305)		
HS6ST1	4	3	3.23E-06	3.01E-05
LRRIQ3	12	88	1.66E-07	4.65E-06
EAP1	8	39	1.42E-06	1.99E-05
IGSF10	13	292	0.0029	0.020
SEC24A	3	17	0.0051	0.029
ZNF560	2	6	0.00823	0.038
FTO	3	29	0.0188	0.058
LGR4	8	172	0.0155	0.058
CYFIP1	2	10	0.01852	0.058
TTF1	7	96	0.023	0.064
THBS4	3	33	0.02574	0.066
INHA	3	39	0.0384	0.090
SYPL2	5	114	0.04472	0.096
ELFN2	2	22	0.06745	0.135
SETBP1	2	25	0.0829	0.155
TBX6	4	102	0.1204	0.211

FANCL	3	78	0.175	0.288
ZNF266	2	54	0.236	0.367
HTR3D	2	60	0.3035	0.433
ICAM3	1	20	0.315	0.433
BCLAF1	4	393	0.3249	0.433
ARHGEF16	3	100	0.4329	0.551
CUX1	1	39	0.5141	0.626
CPZ	2	196	0.5867	0.684
PANX3	2	120	1	1
WDR25	2	126	1	1
ERCC4	3	202	1	1
LRRN1	0	4	1	1

Minor allele frequency (MAF) data was retrieved from the ExAC Browser (Exome Aggregation Consortium (ExAC), Cambridge, MA: http://exac.broadinstitute.org, accessed October 2015). *by both SIFT (1) and Polyphen2 (2). P value calculated by Fisher's exact test. **Benjamini & Hochberg method (3)

Appendix Table S2: Auxological and Pubertal Data of DP patients from the cohort with potentially pathogenic mutations in *IGSF10* as compared to patients without mutations in *IGSF10.*

	Males		
	DP patients with *IGSF10* mutations (mean±SD)	DP patients without *IGSF10* mutations (mean±SD)	
	n=18	n=64	
Birth weight (kg)	3.6±0.6	3.7±0.4	ns
Birth length (cm)	50.6±1.9	50.9±1.2	ns
Age at G2 stage (yrs)	15.2±0.8	15.2±0.9	ns
Age at "take off" (yrs)	15.0±1.3	14.9±0.7	ns
Age at PHV (yrs)	16.3±1.2	16.1±1.0	ns
Adult height (cm)	177.0±5.9	179.3±7.0	ns
	Females		
	DP patients with *IGSF10* mutations (mean±SD)	DP patients without *IGSF10* mutations (mean±SD)	
	n=8	n=125	
Birth weight (kg)	3.7±0.2	3.6±0.5	ns
Birth length (cm)	50.3±2.3	50.0±2.2	ns
Age at B2 stage (yrs)	13.5±1.0	13.8±1.0	ns
Age at "take off" (yrs)	13.3±0.7	12.5±1.1	ns
Age at PHV (yrs)	14.5±0.7	13.4±1.0	ns
Adult height (cm)	168.5±5.4	165.1±6.5	ns

Tables are divided into male and female and compare auxological data and pubertal timing of those DP patients with potentially pathogenic mutations in *IGSF10* (p.Arg156Leu, p.Glu161Lys, p.Glu2264Gly and p.Asp2614Asn) with those without mutations in *IGSF10.* All comparisons were found to be non-significant (ns) by Student's *t* test (2-tailed); G2 – Tanner genital stage 2; B2 – Tanner breast stage 2; "take off" – age at start of pubertal growth spurt; PHV – peak height velocity.

Appendix Table S3: Frequency of *IGSF10* variants in the hypogonadotropic hypogonadism (HH) cohort.

HH cohort (n=334)			**ExAC database (n=32200)**			**P value**
Total number of rare and predicted damaging variant alleles*	Allele Frequency	Total Prevalence	Total number of rare and predicted damaging variant alleles*	Allele Frequency	Total Prevalence	
34	5.1%	10.2%	2314	3.5%	7%	0.0329

Minor allele frequency (MAF) data for the European control subjects was retrieved from the ExAC Browser (Exome Aggregation Consortium (ExAC), Cambridge, MA: http://exac.broadinstitute.org, accessed October 2015). P value calculated by Fisher's exact test. *rare variants with MAF <2.5% and predicted damaging by both SIFT and Polyphen2

Appendix Table S4: Clinical characteristics of two patients with hypothalamic amenorrhea (HA) and HA-equivalent with a shared rare variant in *IGSF10* (NM_178822: c.7353_7354insATCA: (rs570110855) p.L2452fs).

Characteristic	Patient 1	Patient 2
Clinical Characteristics		
Gender	Female	Male
Age (yr)		
At menarche	Unknown	-
At diagnosis	25	29
BMI at diagnosis	25 (low body fat to muscle ratio)	18
Predisposing factors		
Weight loss	No	Yes
Excessive exercise	Yes	No
Subclinical eating disorder	No	Yes
Fertility Status	Failed attempt at conception off treatment; undergoing gonadotropin therapy	No attempt at conception
Family history of hypothalamic amenorrhea	No	No
Radiological Findings		
MRI	Normal pituitary	Normal pituitary
Biochemical Findings		
LH (U/L)	5.0	0.6
FSH (U/L)	5.9	4.1
Estradiol (E_2) (pg/mL)	<15	-
Testosterone (ng/mL)	<0.3	0.8
Prolactin (mU/L)	129	111
TFTs	Within normal range	Within normal range
AMH	40.3	-

The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters; LH- lutenising hormone; FSH – follicular stimulating hormone; TFTs – thyroid function tests, comprising of free T4 and thyroid stimulating hormone; AMH – anti-mullerian hormone

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3. Benjamini Y, Drai D, Elmer G, Kafkafi N, and Golani I. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res.* 2001;125(1-2):279-84.