EXACTAS UBA

Instituto de Biociencias, Biotecnología y Biomedicina

Desarrollo de paneles de secuenciación aplicados al hipopituitarismo congénito

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Jornadas de Genómica Clínica
2022

Descubriendo genes:

*Diagnóstico Molecular

*Predicción del curso de una patología

*Selección de tratamiento

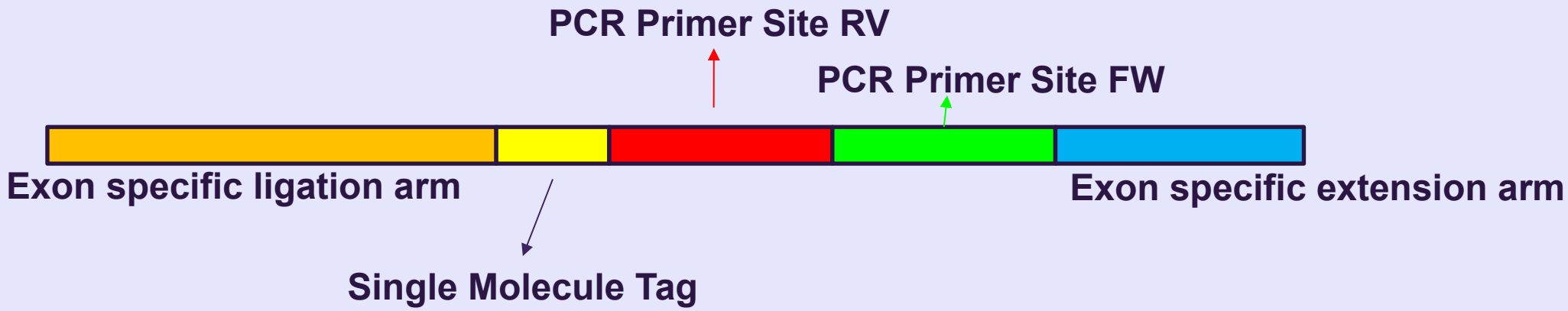
*Identificación de nuevas drogas

*Asesoramiento familiar

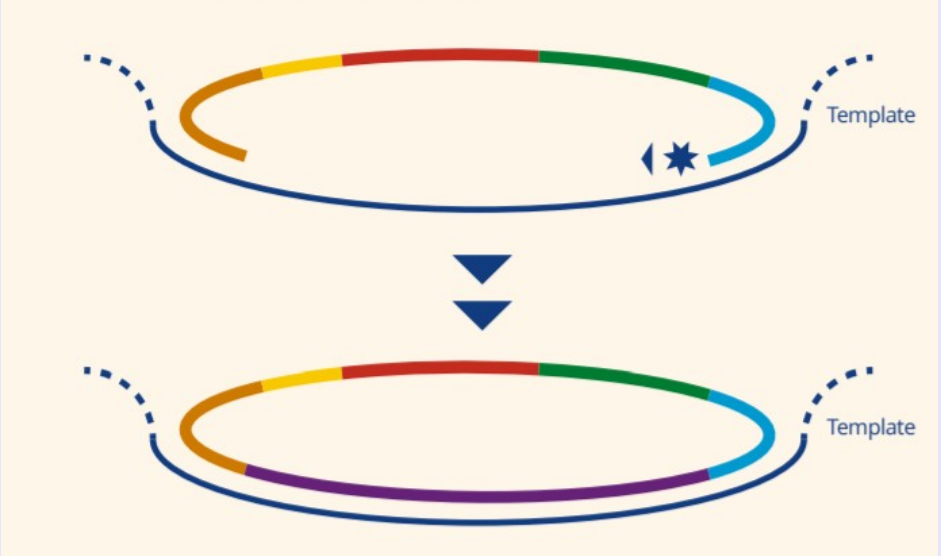
Conocimiento de la fisiopatología de la enfermedad!

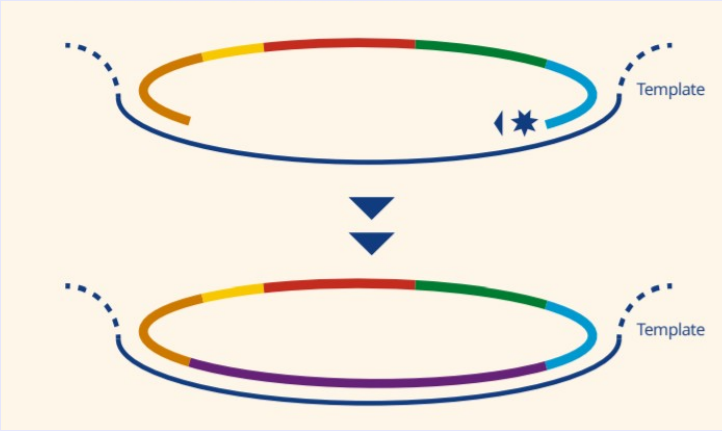
Approach: smMIPS

small molecule Molecular Inversion Probe Sequencing



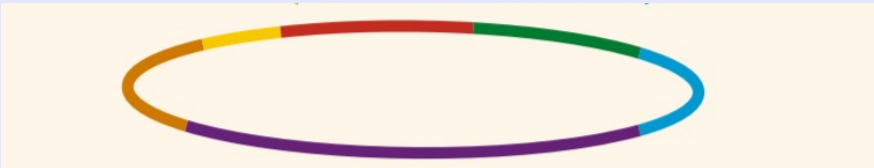
1) Hybridization of the smMIP to the target exon, gap-filling and ligation.





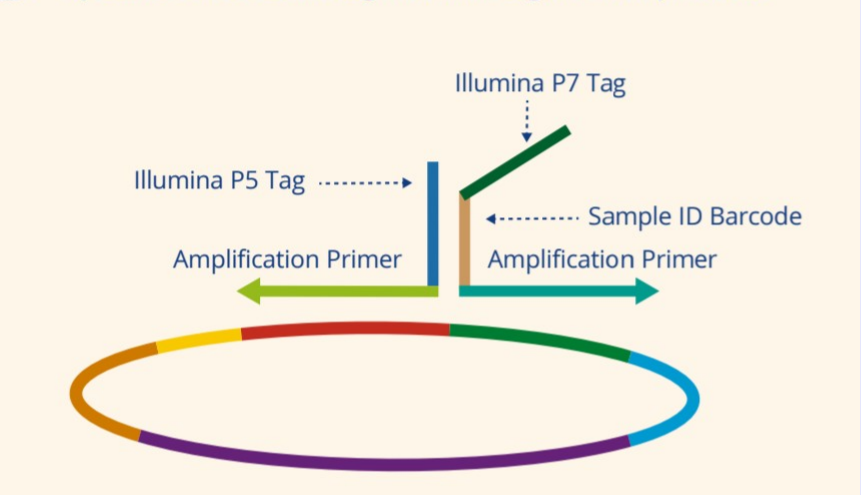
Exonuclease

2) Removal of all non-circular DNA (non ligated smMIP molecules and template DNA)

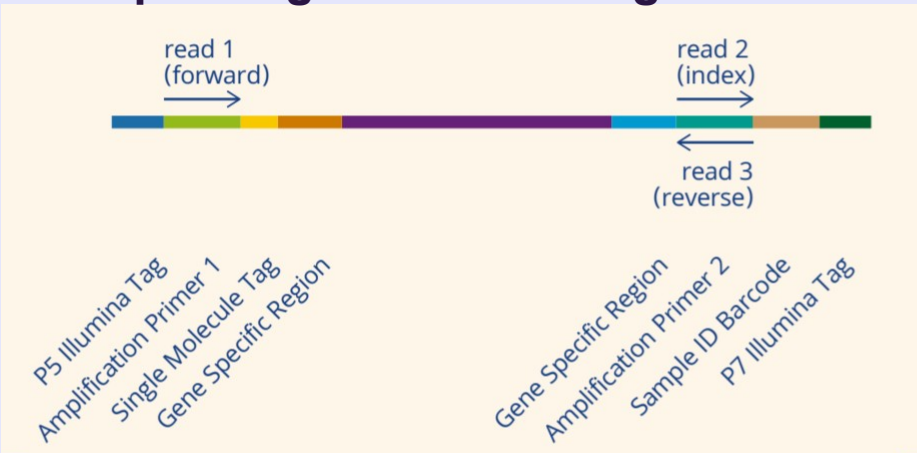


Pool samples from up to 200 people for 1 sequencing run

3) Amplification,
with tailed primers to include sample specific barcode and Illumina tags in final



Final, sequencing-ready product, including sample specific barcode and unique single molecule tag



Ventajas del uso de paneles basados en smMIPS

- MIPS capture 10^2-10^4 targets (e.g., exons) in a single reaction
- No specialized instrumentation required
- Probes are synthesized individually or on a DNA microarray
- Reagent+seq cost for typical (~200 gene) panel ~ $15
- Molecular barcodes: detect dup reads, correct errors

Ventajas del uso de paneles basados en smMIPS

Per sample (sample) and per genotype (genotype) costs ($).

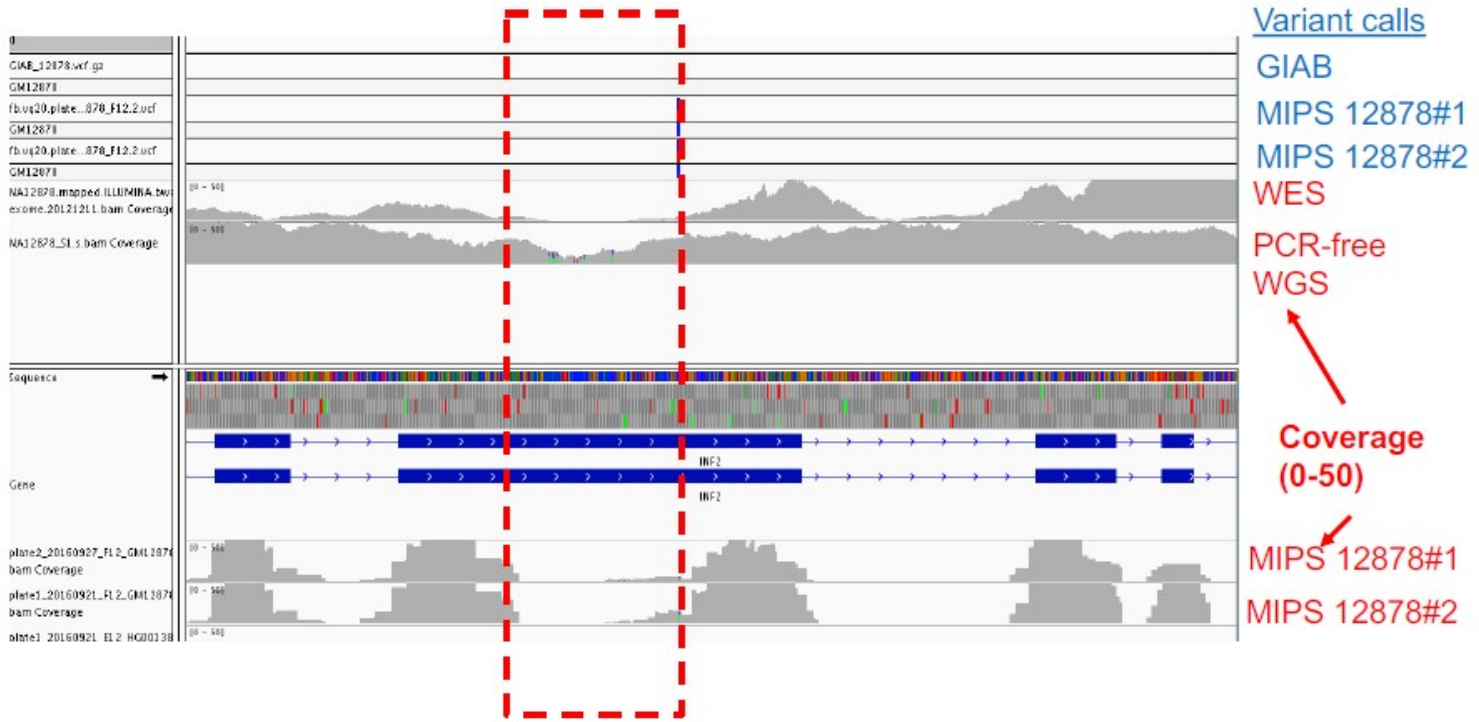
N samples	N MIPs					
	100		1000		5000	
	sample	genotype	sample	genotype	sample	genotype
100	16.6	0.166	98.5	0.098	462.5	0.092
1000	8.3	0.083	16.5	0.017	52.9	0.011
10000	7.5	0.075	8.3	0.008	12.0	0.002

Open in a separate window

These costs include probe synthesis ($9.1 per MIP, does not depend on the number of samples) and reagents for pool phosphorylation, hybridization, gap filling, ligation and PCR amplification ($7.4 per sample, does not depend on the number of MIPs).

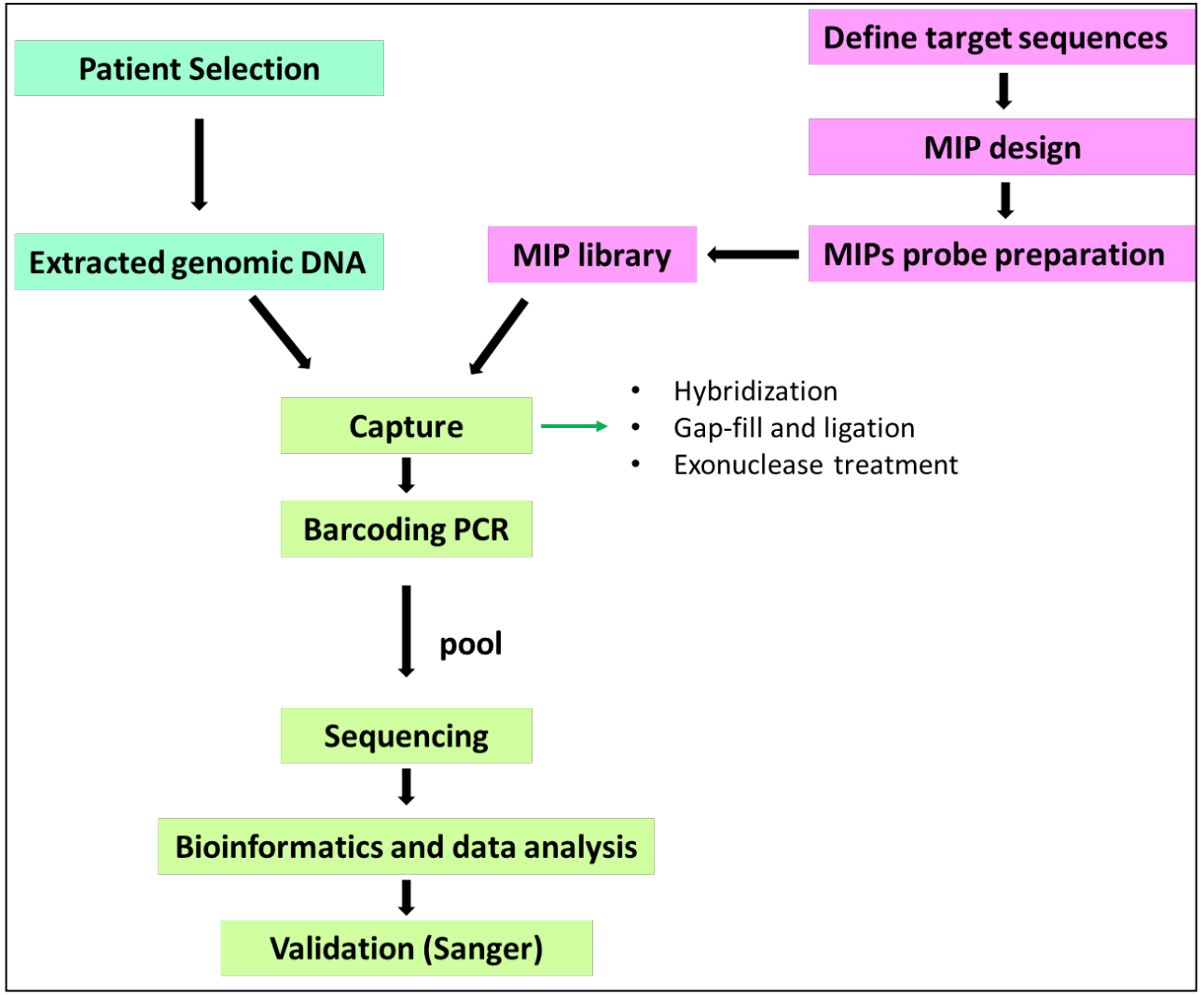
Lower performance at certain targets

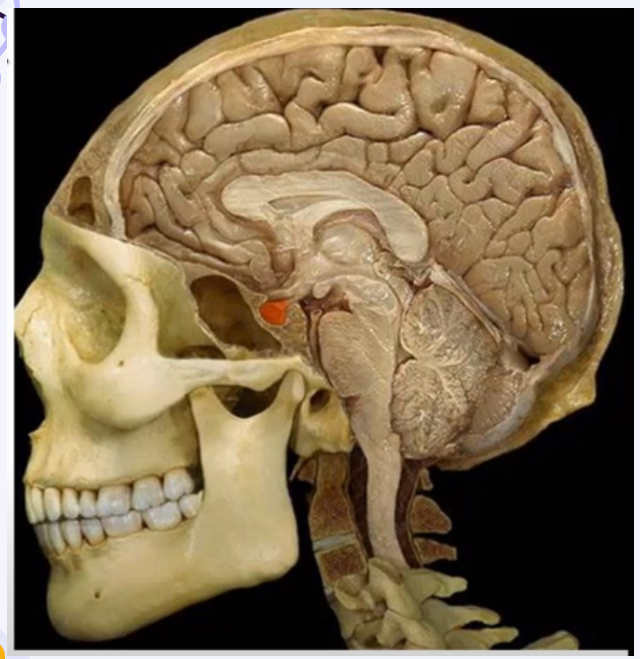
- Among positions with low MIPS coverage (≤10X):
  63.1% of target bases are low in ExAC
- Among other positions, only 7.2% of bases low in ExAC
- Some may be hard (~80% G+C or mono-nuc tracts)
- The rest may be recoverable, with "rescue" probe design



Workflow illustrating the design of Molecular Inversion Probes and laboratory procedures

QC:
concentration,
purity
50 ng/ul





Aplicación a una patología en concreto

Anomalias hipofisarias congénitas

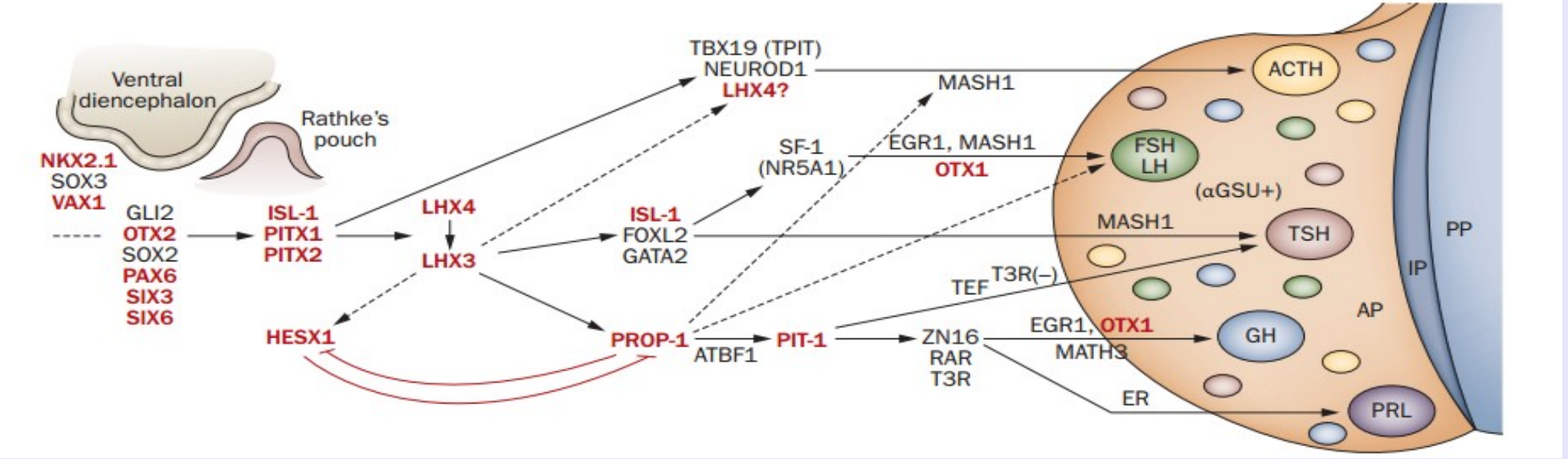
Anomalías del desarrollo de la hipófisis

~1:4,000 Nacimientos vivos

Severo

Leve

Holoprosencefalia (HPE):		GLI2		
Displasia Septo-óptica (SOD):	HESX1			FGF8
Def. comb. de hormonas hipofisarias CPHD):	HESX1	GLI2	SOX3	FGF8
Hipogonadismo hipogonadotrófico:	HESX1	GLI2	SOX3	FGF8
Def. aislada de GH (IGHD):	HESX1		SOX3	FGF8



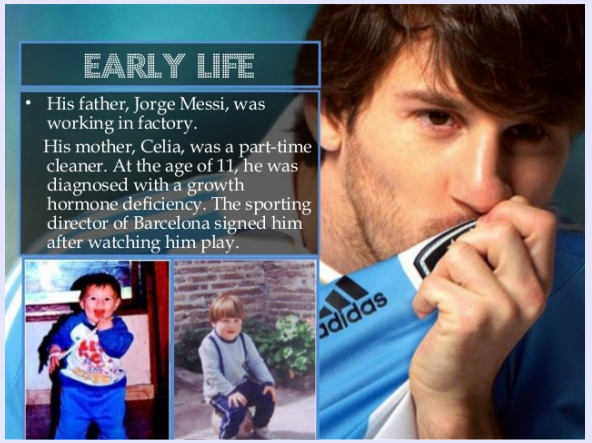
Nat. Rev. Endocrinol. 7, 727–737 (

Deficiencia hipofisaria o hipopituitarismo

Congenital

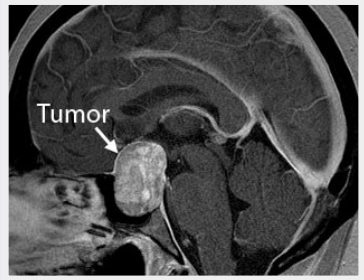
1/4000 births

- Isolated GH Deficiency (IGHD): *GH, GHR*
- Combined Pituitary Hormone Deficiency (CPHD)
  - transcription factor defects

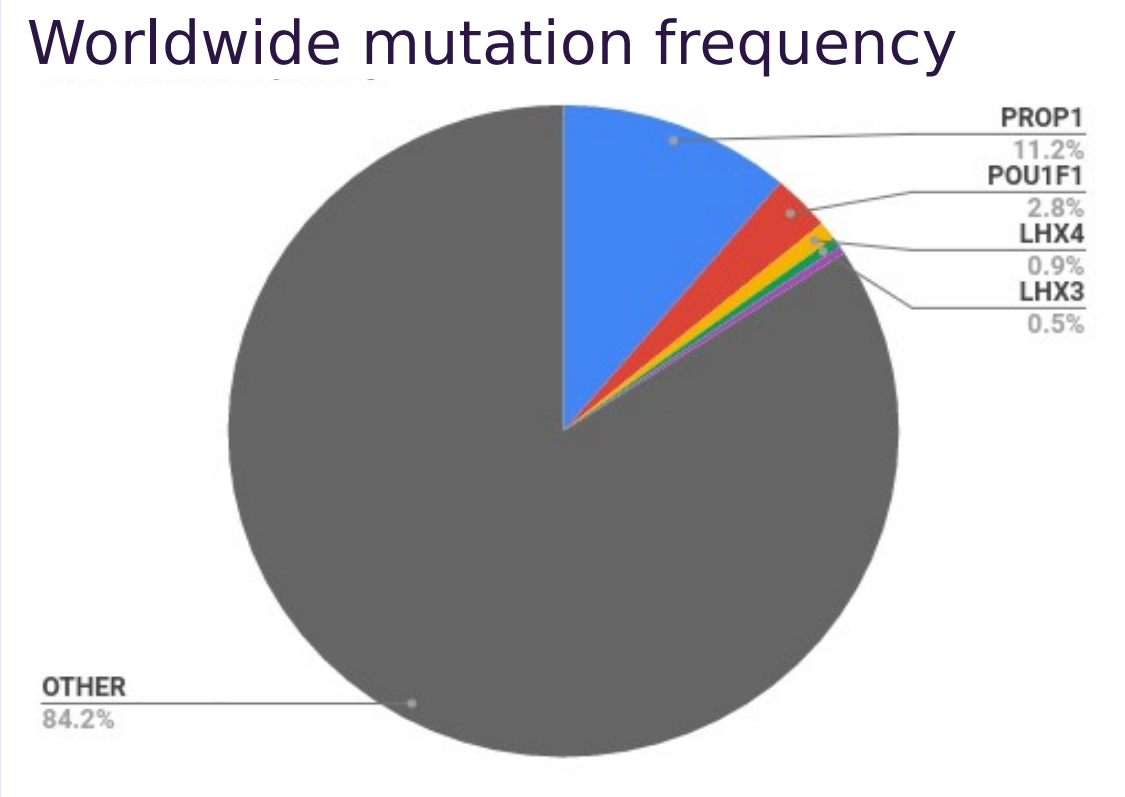


Acquired

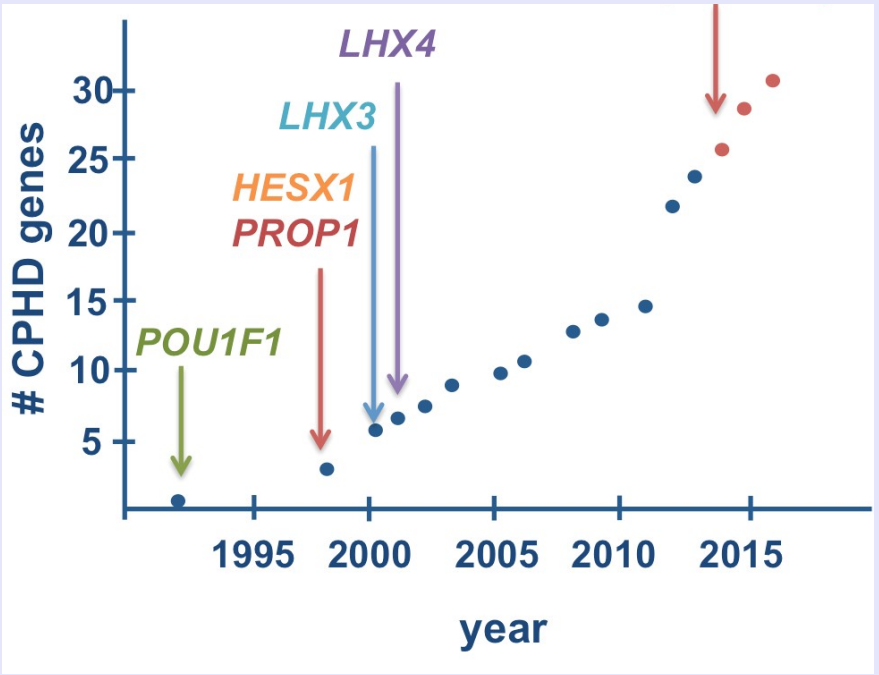
- craniopharyngioma (childhood)
- pituitary adenomas
- head trauma
- radiation therapy (head)
- autoimmune inflammation – hypophysitis
- infarction – Sheehan syndrome, pituitary apoplexy



Genes asociados a hipopituitarismo



Secuenciación
De Exomas



de Rienzo et al., Clin Endo, 83:849-60, 2015.
Fang et al., Endocrine Reviews 37:636-675, 2016.

Panel de genes

Genes en los cuales mutaciones fueron identificadas en pacientes con deficiencias de hormonas hipofisarias y enfermedades relacionadas
AIP, ARNT2, BMP4, DUOX2, EIF2B5, ELP4, FGF8, FGFR1, FOXA2, GH1, GHR, GHRH, GHRHR, GHSR, GLI2, GNRHR, GPR161, HESX1, HHIP, HMGA2, IGSF1, IRS4, KAL1, LHX3, LHX4, Nkx2-1, NR0B1, OTX2, PAX6, PITX2, POU1F1, PROP1, PROKR2, SEMA3A, SLC15A4, SOX2, STAT5b, SOX3, TBX19/, TPIT, WDR11, ZSWIM6
Genes candidatos, basados en experimentos en modelos animales
ARX, ACVRL1, BMP2, BMP7, CDH1, CDH2, CDKN1B, DIO2, DMXL2, FGF10, GLI3, HNF1A, HNRNPU, INSM1, ISL1, NKX2-6, NOG, NRP2, OTX1, RAX, SDHA, SHH, SIX3, SMOC2, TCF7L1, TGIF1, WNT4, WNT5A, ZEB2
Genes recientemente reportados en pacientes con deficiencia hipofisaria
ACP5, AKT3, B3GAT3, BTK, CDK6, CDON, CUL7, EIF2S3, IARS2, IFT172, IGFALS, KIAA0753, NFKB2, OBSL1, PNPLA6, POLR3A, PROK2, RBM28, RNPC3, ROBO1, TCF7L2, TMEM67, XYLT1
Proteínas interactuantes con PROP1 (resultados preliminares de nuestro laboratorio)
CTNNB1, GABPA, GTF2I, NFIB, PBX1, SMARCA5, STAT3, THAP11, ZBTB20

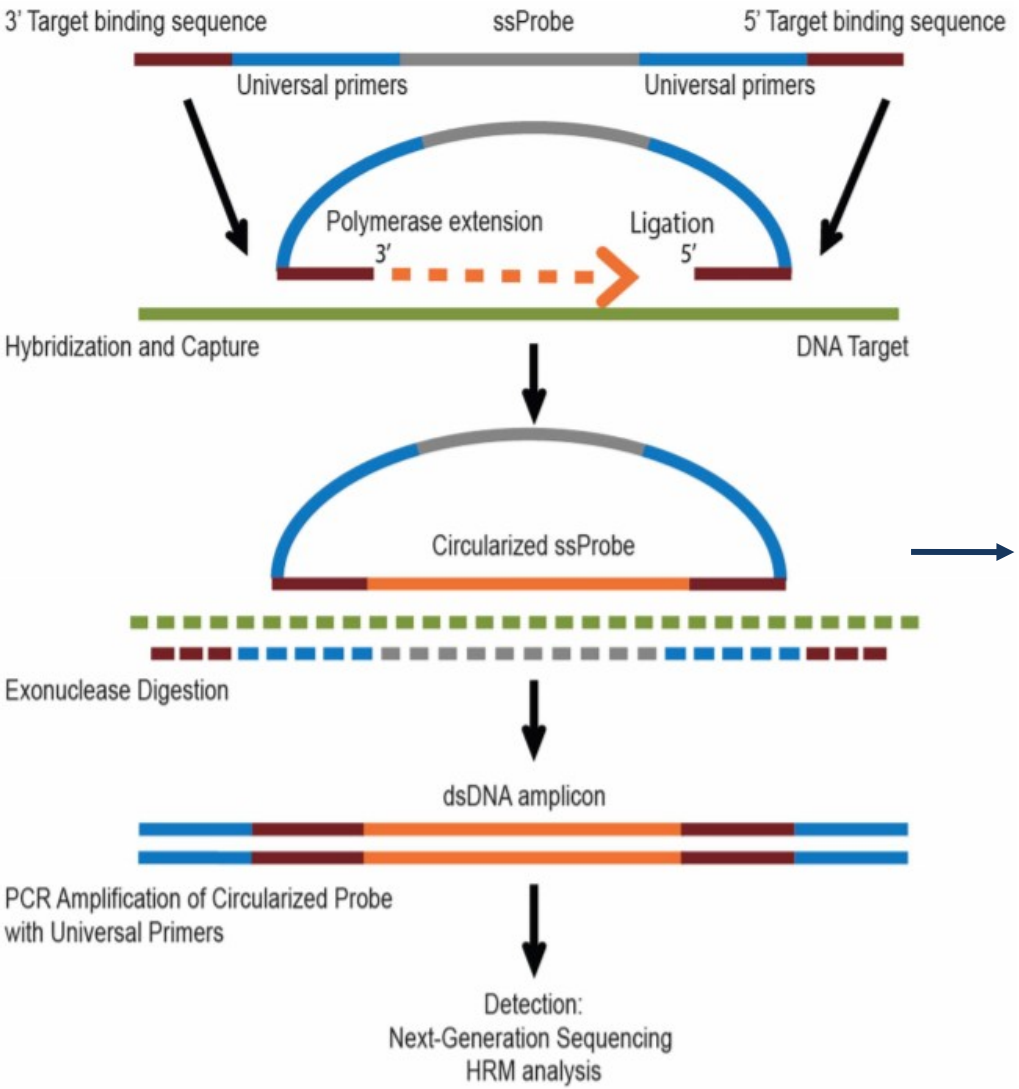
104 Genes, 12k sondas

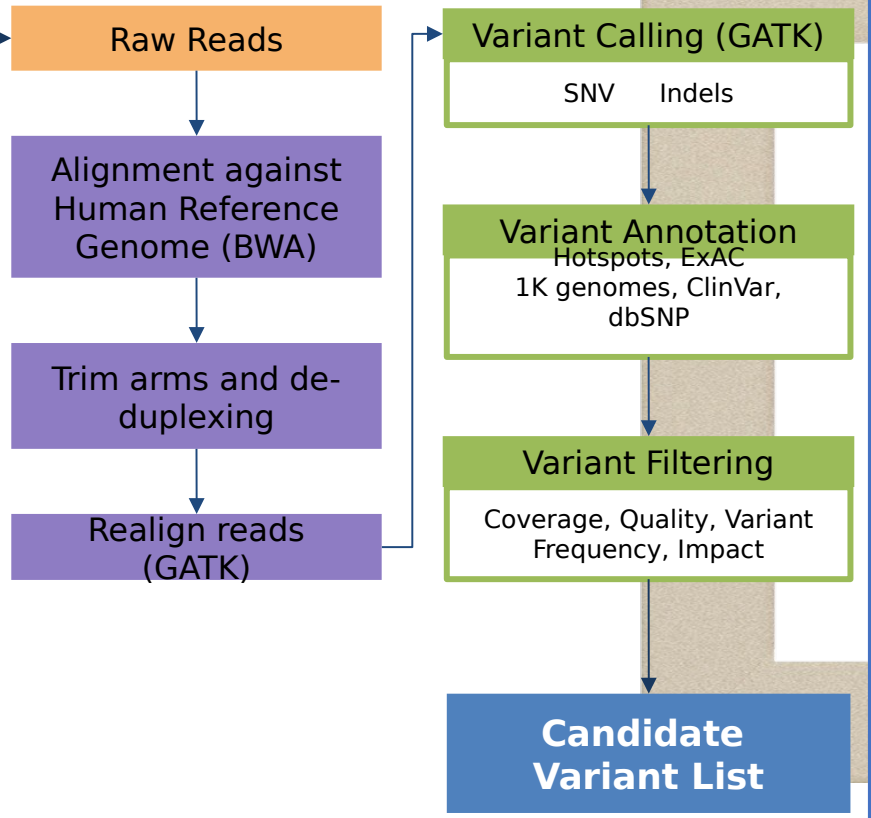
Pacientes incorporados al estudio

Muestras	180
Casos	95
Esporádicos	93
Familiares	2
Edad Diagnóstico	8,9 +- 8,9 años
Género	
Masculino	58 (61%)
Femenino	37 (39%)
Diagnóstico principal	
IGHD	26 (27%)
CPHD	65 (68%)
Otros	
Displasia Septo óptica	8
Holoprosencefalia	2
Deficiencia Hormonal	
GH	76 (80%)
ACTH	46 (48%)
TSH	55 (58%)
Gonadotropinas (LH, FSH)	12 (13%)
PRL	2 (2%)

Resonancia magnética	
Adenohipófisis	
Hipoplasia	60
Ausencia	3
Normal	7
Neurohipofisis	
Ectópica	42
Ausencia	7
Normal	14
Tallo	
Ausencia	24
Delgado	11
Interrumpido	5
Normal	18
Doble	1
Urogenital	
Micropene	6
Criptorquidismo	6
Hipogonadismo	3
Retraso puberal	2
Pubertad precoz	1

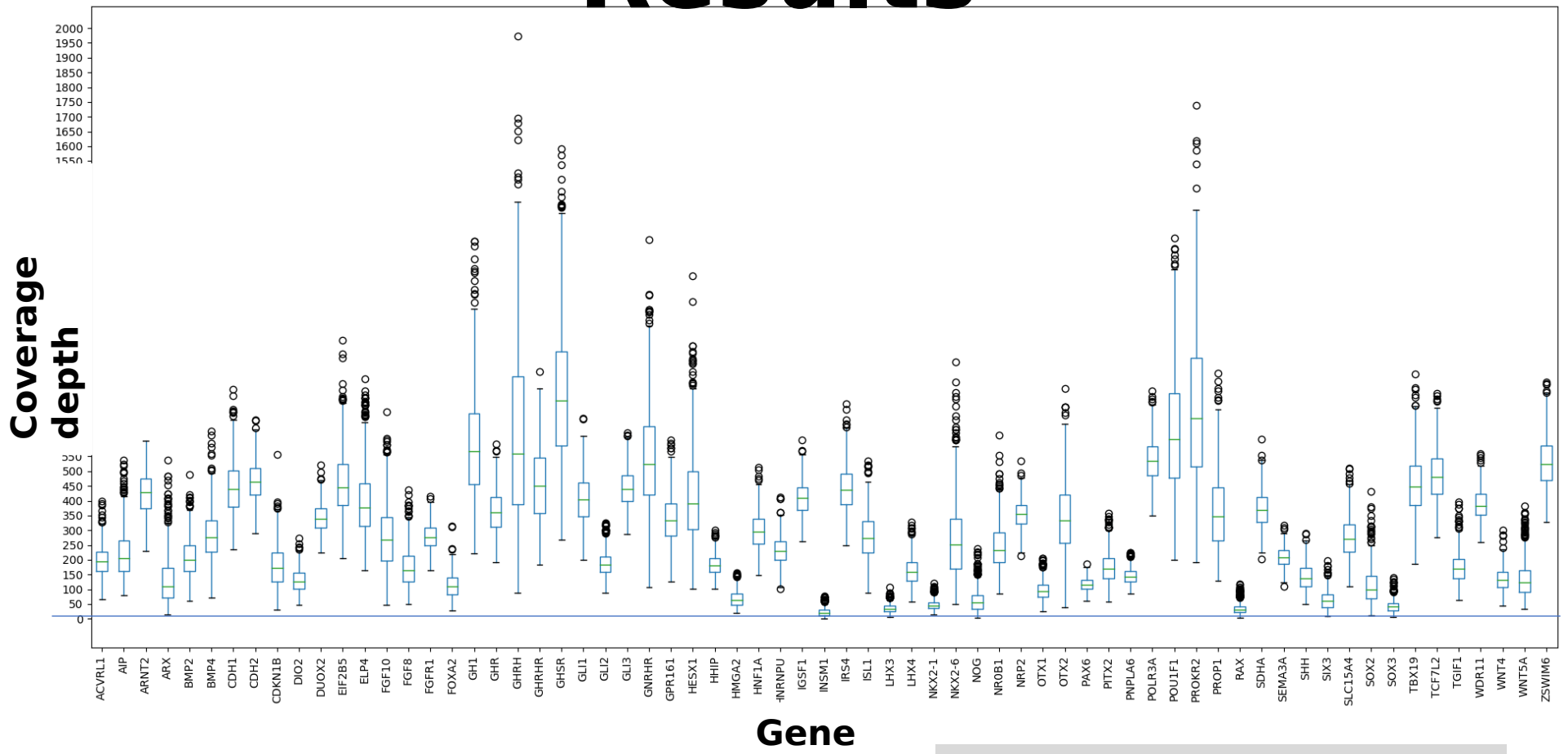
+ 170 pacientes secuenciados con un panel de 67 genes



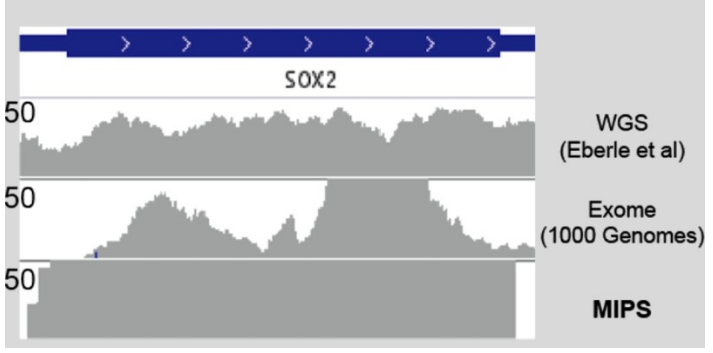


Adopted from Stefan CP,et al. Scientific Reports. 2016;6:25904.

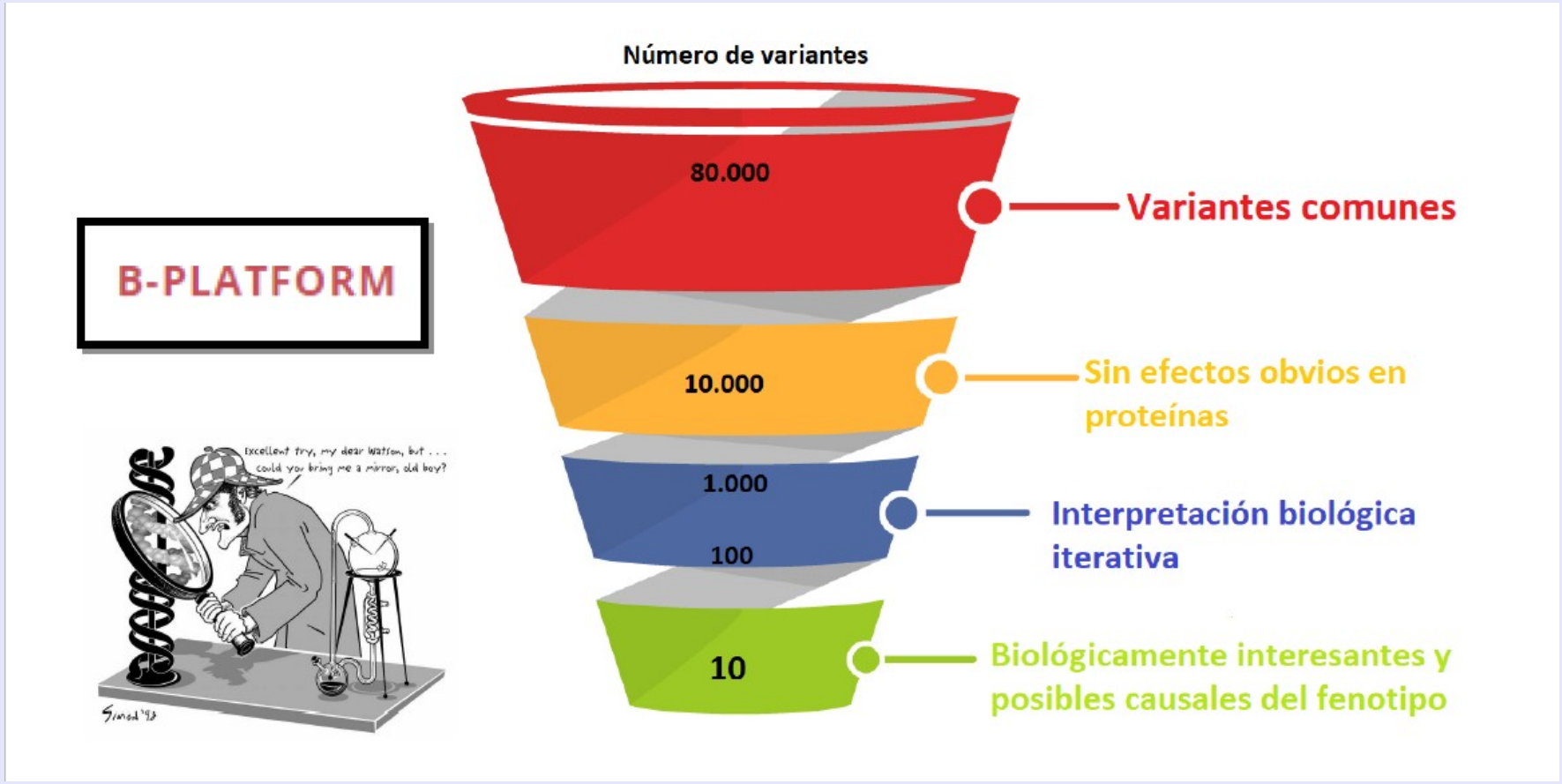
Panel: Sequencing Results



99.54% SNP/indel variant sensitivity, overall genotype concordance of >99.6%



Como reducimos el numero de variantes



Evaluation of Candidate Variant List

BITGENIA
100 EXOMAS

1. Appropriate population controls:
   - ✓ Gnomad
   - ✓ Exac
   - ✓ 1000G
   - ✓ Private database Argentina

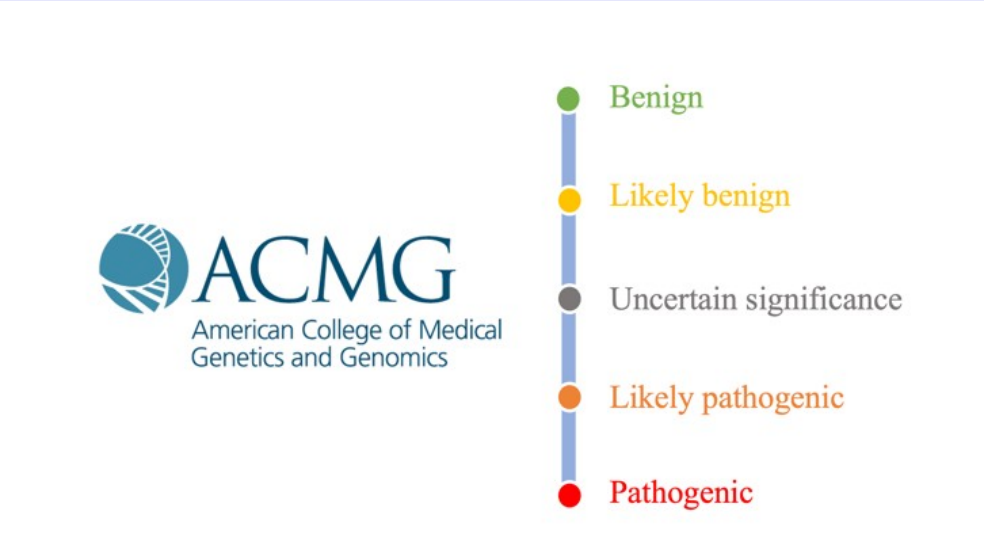
variants
extremely rare or not
observed MAF <0.1%

2. Software predictions of pathogenicity:
   - ✓ SIFT
   - ✓ PolyPhen
   - ✓ CADD
   - ✓ Mutation Tester

3. Segregation in the family

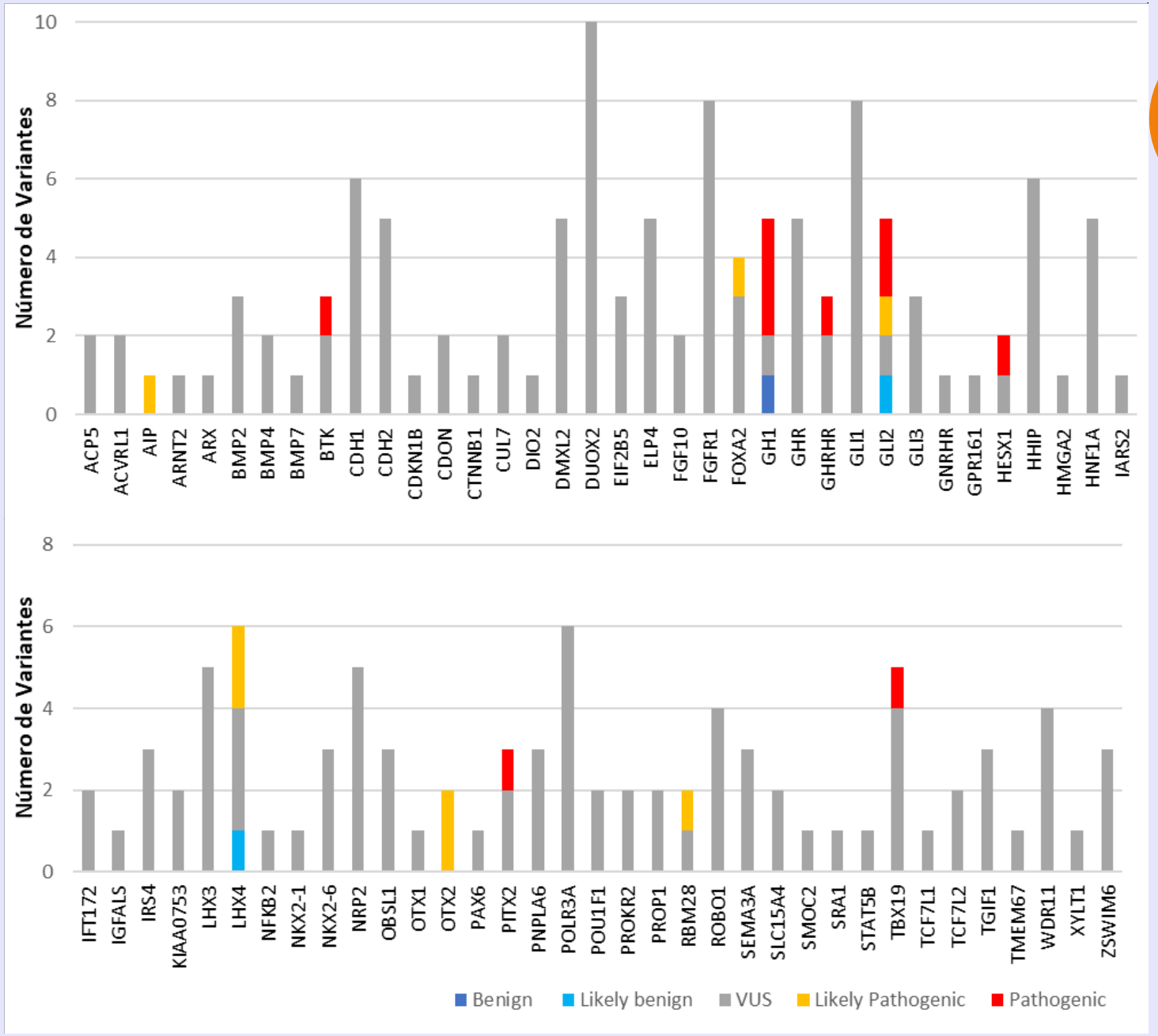
4. Observation in unrelated, affected individuals

Clasificación de variantes



	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other		Reputable source w/out	Reputable source			

205 variantes en 149 probandos individuales



Que datos nos importan de una variante?

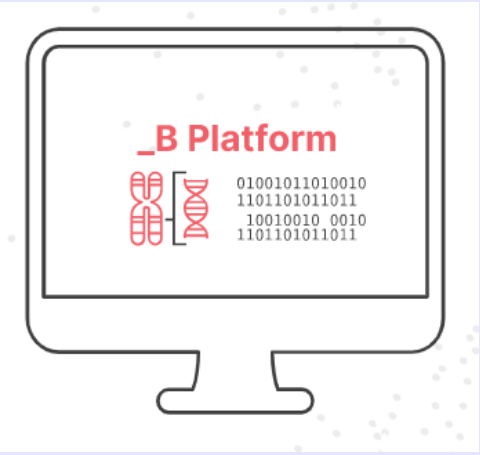
Clasificación segun ACMG

Chr:position	Gene	Change	Impact/Effect	ACMG	Frequency	Evidence	Samples Information
21:27130403	GABPA	C > CA c.637dupA p.(Thr213fs)	High frameshift variant	Source:Intervar Likely pathogenic PVS1 PM2	Esta en la población sana?	Gene: GABPA OMIM ClinGen GeneCards gnomAD Variant: gnomAD M.Taster	POS197_Proband P Het DP:66/308 |GQ:99 |FS:28.684 |QUAL:1502.73 |Filter:PASS POS196_Father F Het DP:52/229 |GQ:99 |FS:14.789 |QUAL:1216.73 |Filter:PASS View Samples

Posición en el cromosoma

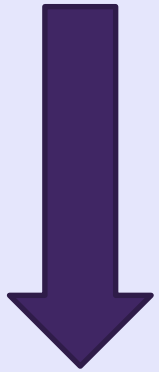
Cambio en el ADN y en la proteina

Efecto Molecular



Una vez hallada la/s variante/s candidata/s

¿Como llevamos a cabo la interpretación biológica y clínica?



Busqueda en Bases de Datos

Que bases de datos se pueden consultar?

1 Informacion propia del gen o patologia

OMIM UniProt ENSEMBL
.....

2 Informacion ya documentada de la variante

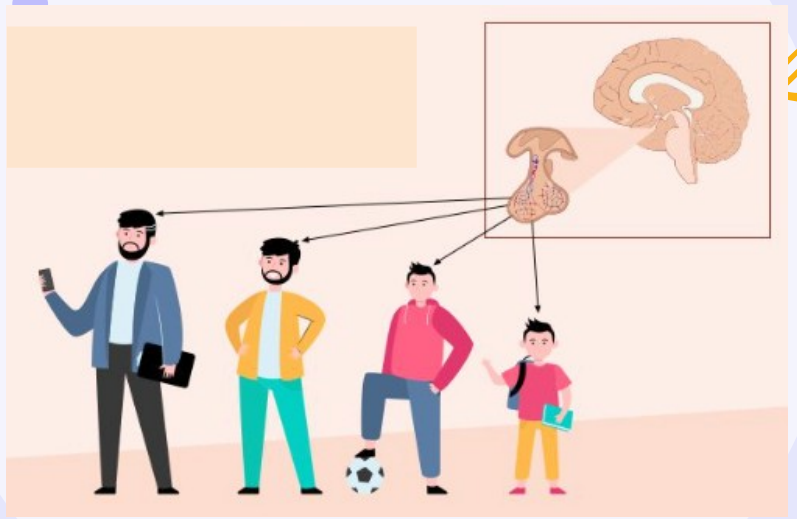
CLINVAR dbSNP
gnomAD

3 Predicciones de patogenecidad y conservación

CADD MutationTaster
Polyphen...

4 Base de datos

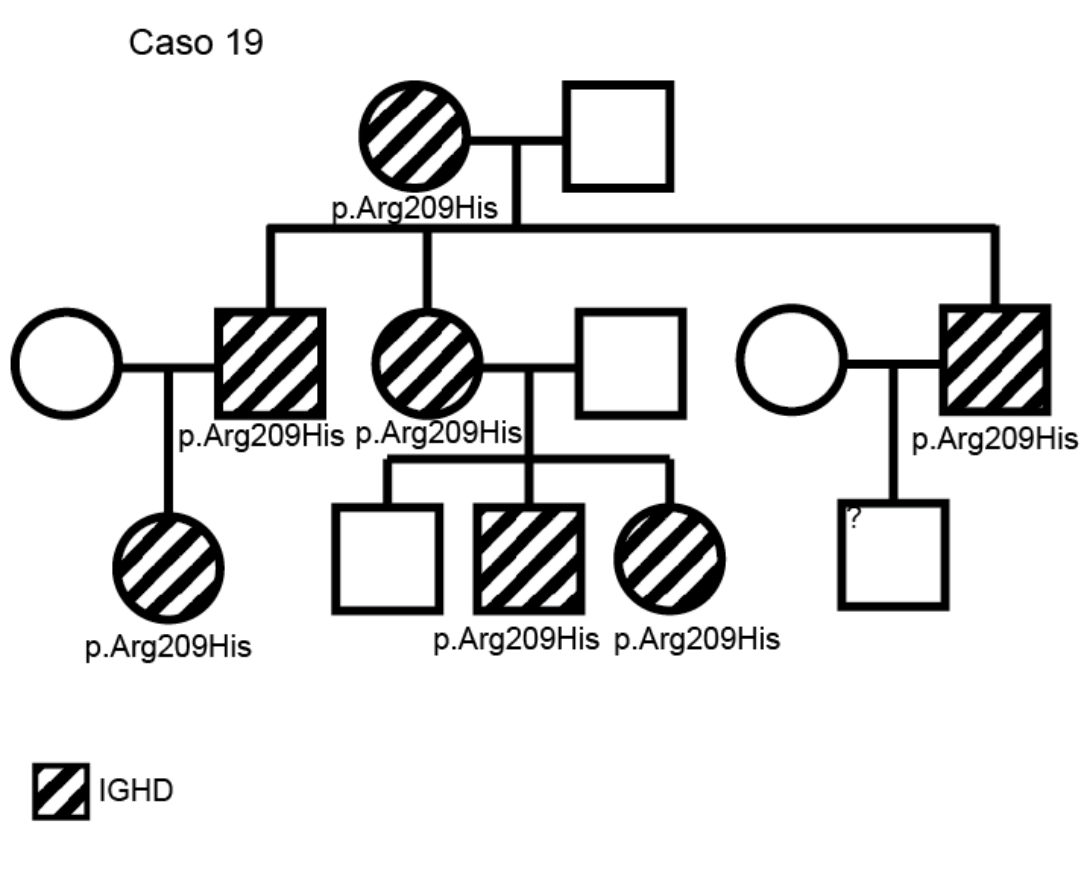
UK BioBAnk Genbass

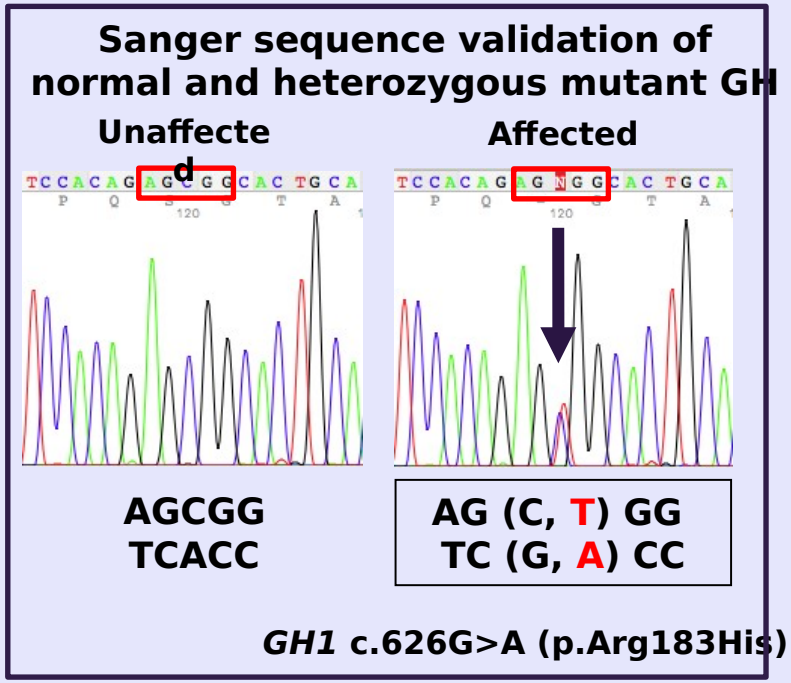


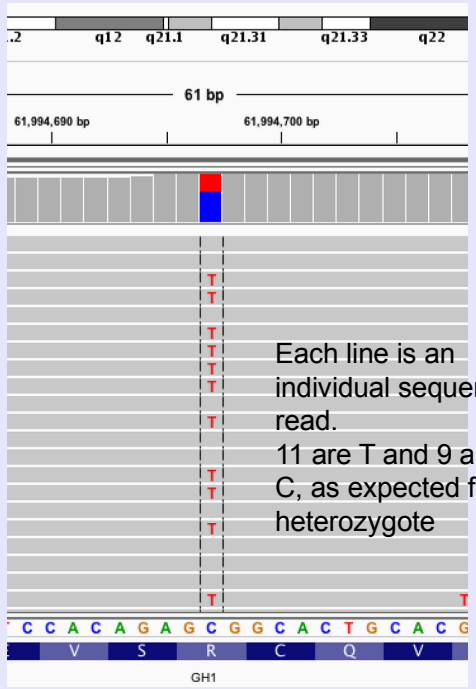
Caso Clinico 1

Panel

Deficiencia de Hormona de Crecimiento en un caso familiar







Mol Genet Genomic Med. 2018 Jul; 6(4): 514–525.

Información clínica de la familia

Table 2: Clinical data of the families evaluated

Family	Gender	Diagnosis	Height (SDS)	Max peak GH (ng/ml)	IGF1 (SDS)	Mutation p.Arg183His
I-2	F	IGHD	-2.5	NA	-8.3	Yes
II-1	M	IGHD	-2.9	2.3	ND	Yes
II-2	F	normal	0.6	NA	NA	No
II-3	M	normal	1.5	NA	NA	No
II-4	F	IGHD	-3.1	NA	-4.8	Yes
II-5	M	IGHD	-3.2	NA	-3.7	Yes
III-1	F	IGHD	-3.5	3.6	-6	Yes
III-3	M	IGHD	-2.9	3.01	ND	Yes
III-5	M	normal	0.35	NA	2.3	no

Abbreviations: NA, not available; ND, not detectable

Normal Range: GH peak value >10

All cases present normal RMN
Rest of hormone levels are normal

GH Arg183His affects secretion of normal GH

Individual with this mutation does have releasable GH stores, but release is severely impaired.

Assay uses tagged proteins in AtT-20 (corticotrope-like) cells

Secretory granules containing R183H-GH are not as effectively exocytosed as those containing wild-type GH

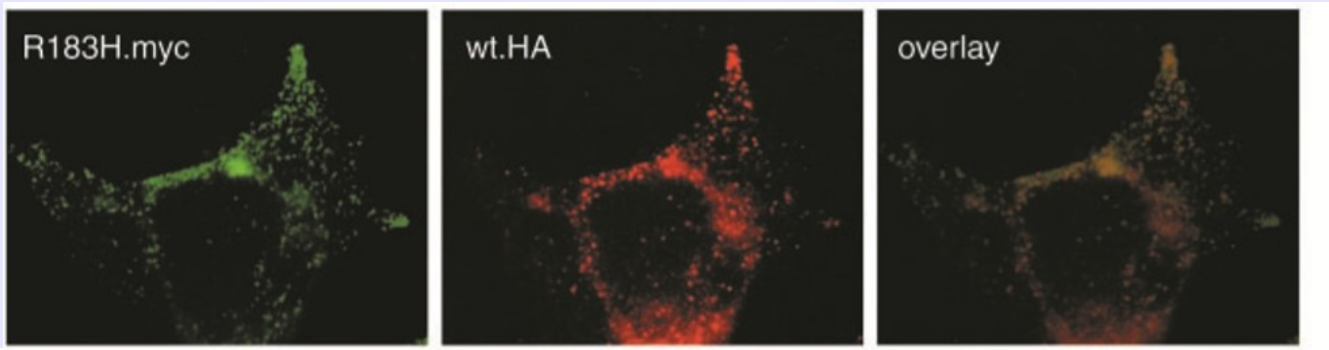


TABLE 2. Effect of forskolin on stable AtT-20 cells expressing individually either normal GH (wt.HA) or R183H mutant GH (R183H.myc) or coexpressing both normal and mutant GH (wt.HAxR183H.myc)

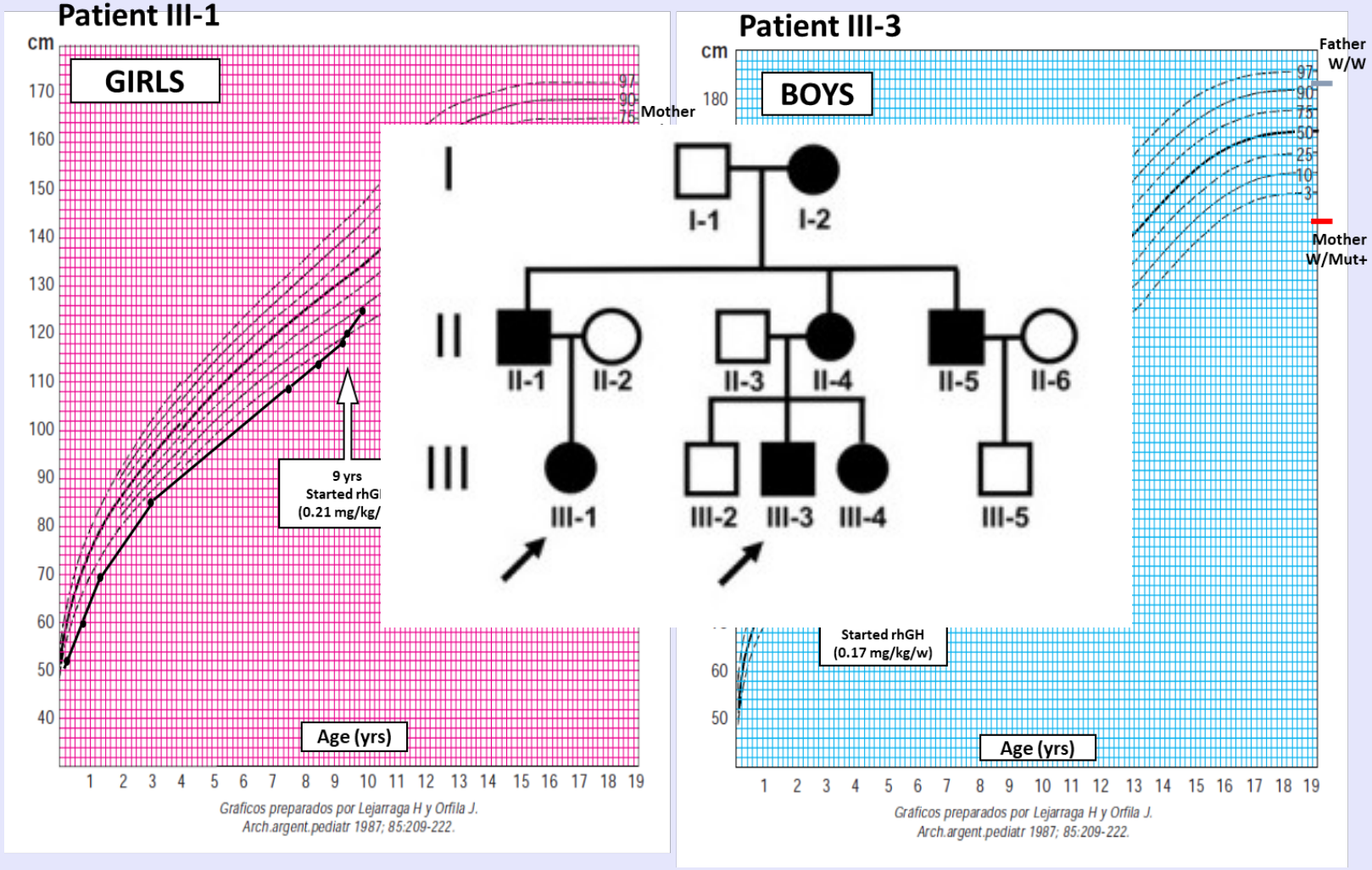
Plasmid	Forskolin	GH		ACTH		Secretion index[a]
		μg/liter	% Basal release	ng/liter	% Basal release	
wt.HA	−	28.00 ± 1.01		2379 ± 99		
	+	34.93 ± 0.34	21.3 ± 3.2	2950 ± 62	24.0 ± 7.2	0.95 ± 0.05[b]
R183H.myc	−	37.61 ± 0.40		2115 ± 66		
	+	44.73 ± 0.90	19.0 ± 3.5	2428 ± 52	15.2 ± 6.3	1.04 ± 0.04[b]
wt.HAxR183H.myc	−	57.18 ± 1.19		1614 ± 125		
	+	63.00 ± 0.14	9.9 ± 4.5[c]	2383 ± 93	49.2 ± 9.7[c]	0.74 ± 0.04
R183H.mycxR183H.myc	−	64.62 ± 0.84		3181 ± 82		
	+	81.00 ± 2.10	25.5 ± 4.9	3854 ± 78	21.4 ± 1.2	1.03 ± 0.03[b]

His18 and His21 residues bind Zn and facilitate GH dimerization

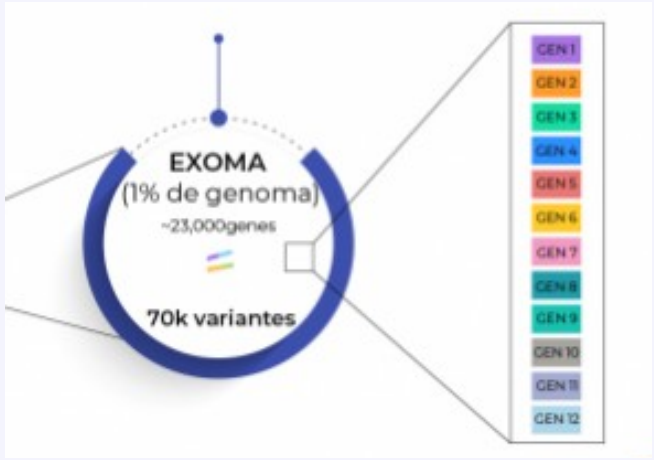
Extra His may impair dimerization or affect conformation

Deladoey et al., *JCEM*, 86:3941-3947, 2001

Curvas de crecimiento



We solved a familial case. We found a dominant mutation *GH1*, p.R183H, in a three-generation pedigree with IGHD.

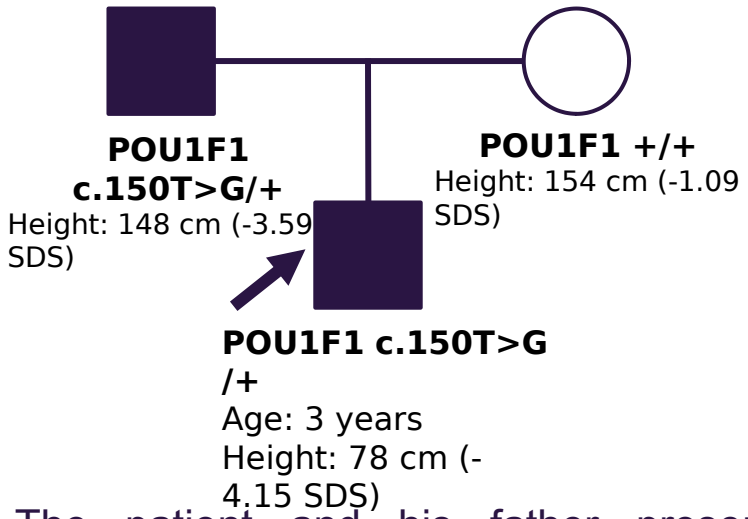


Caso Clinico 2

Familiar. Panel y WES

Genetic variation causes *POU1F1* isoform switching and pituitary hormone deficiency

Familial



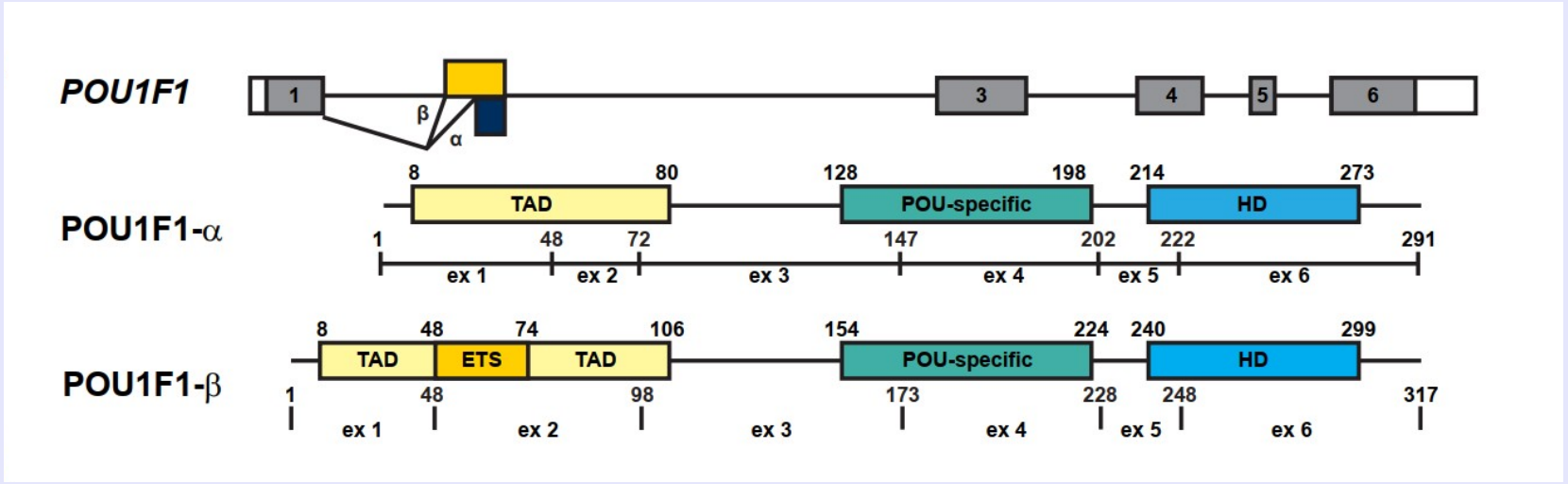
- The patient and his father presented **IGHD.** Normal MRI.
- **WES** for the family revealed a synonymous heterozygous variant (**c.150T>G**, **p.S50S**) in ***POU1F1***

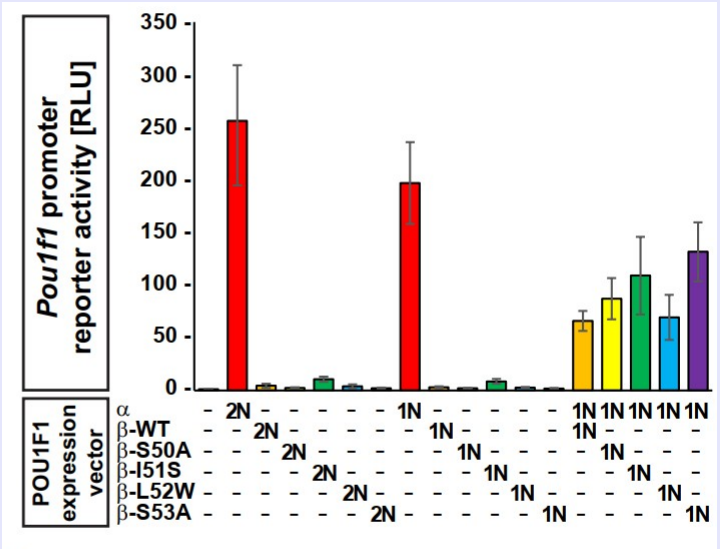
AJHG 2021 Aug 5;108(8):1526-1539. **doi: 10.1016/j.ajhg.2021.06.01**

Alternatively spliced isoforms of *POU1F1* exon 2

Canonical splicing: POU1F1 α
Active isoform

Alternative splicing: POU1F1 β
Repressive isoform





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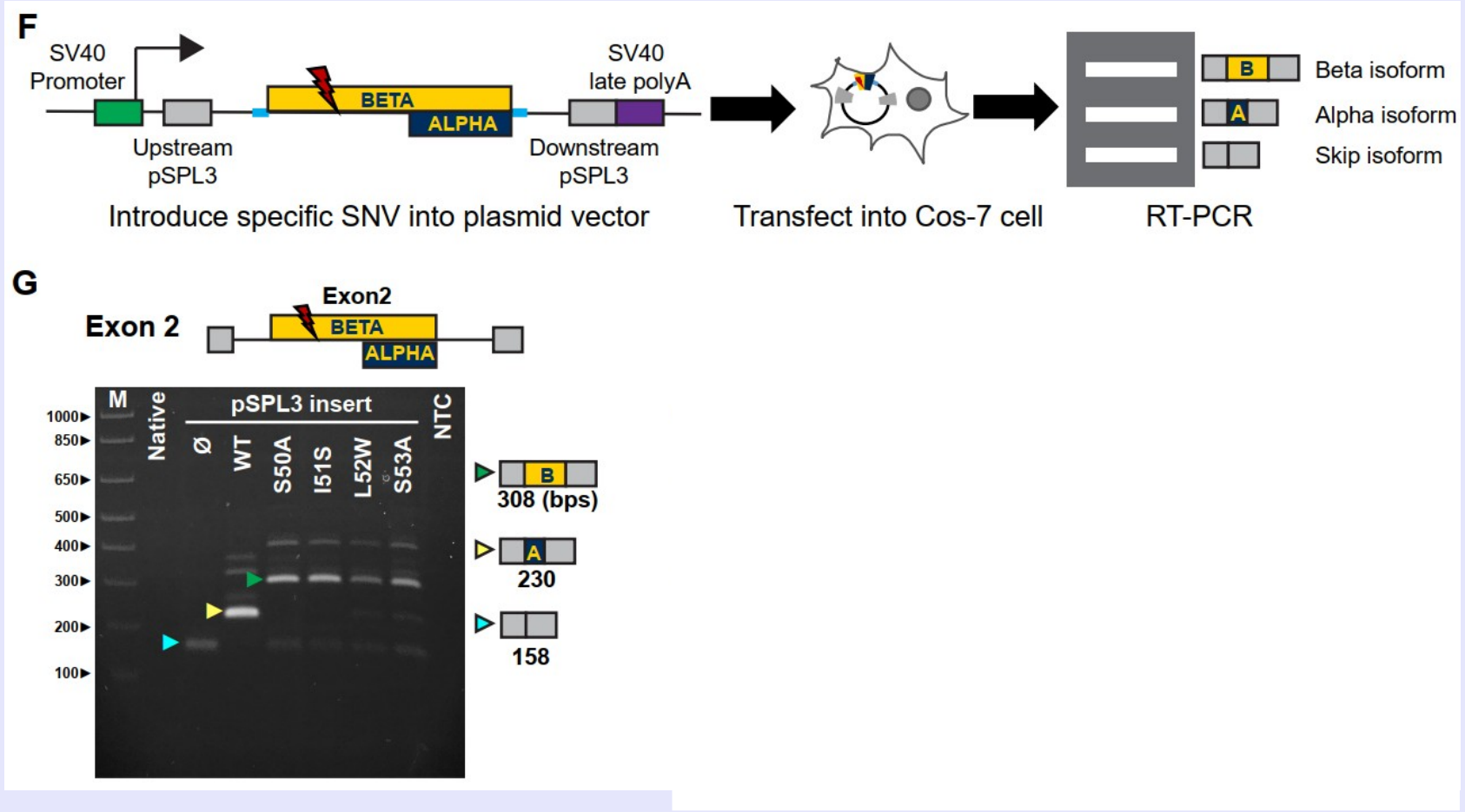
The four POU1F1 beta variants and WT beta repressed POU1F1 alpha activity to a similar degree

But…..these novel patient variants could be impacting splicing and thus increasing inclusion of the normally lowly expressed BETA isoform????

AJHG 2021 Aug 5;108(8):1526-1539. doi: 10.1016/j.ajhg.2021.06.013.

Patient missense variants disrupt normal *POU1F1* splicing to favor the beta isoform

low throughput mini gene assay



Almost no WT expression of BETA but high BETA usage for the four missense patient variants.

Para llevarnos a casa:

- Paneles basados en smMIPS son eficientes y economicos (#muestras # genes). Se los puede actualizar facilmente
- Las estrategias de secuenciaion dependen qué busco, qué tengo. Se complementan
- Equipo multidisciplinario de trabajo

Gracias!
Thank you!

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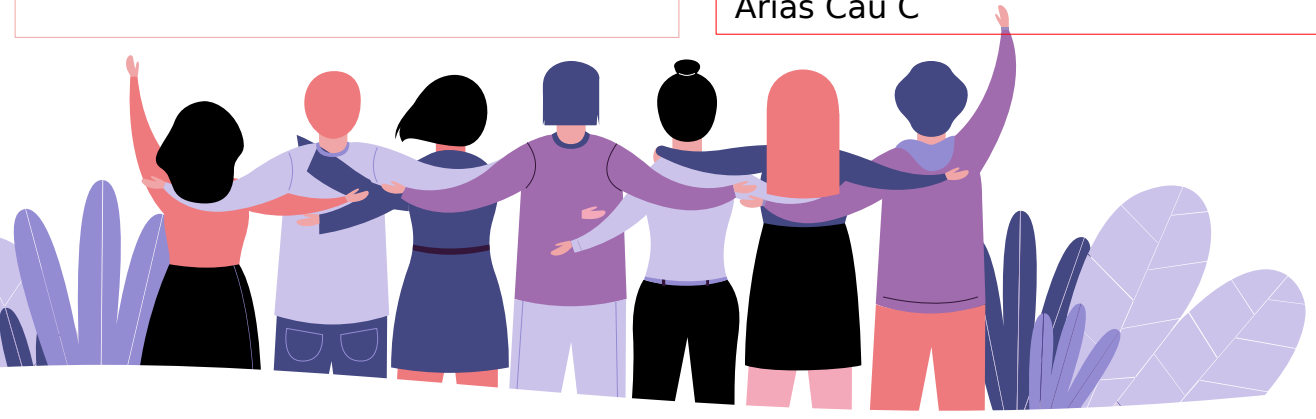

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