2ND SUMMIT
RARE
DISEASES
COPAC

Como alcanzar el diagnóstico de POMPE
La importancia de los estudios genéticos

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MAT-CO-2202507 08/2022

2ND SUMMIT RARE DISEASES COPAC sanofi

Conferencista:

Flor Giliberto

- Bioquímica
- PhD, Universidad de Buenos Aires (UBA)
- Investigadora adjunta CONICET
- Investigadora principal del laboratorio de Distrofinopatías (UBA)
- Docente, Cátedra de Genética. Facultad de Farmacia y Bioquímica, UBA
- Instituto, INIGEM, CONICET-UBA
- Hospital de Clínicas José de San Martín
- e-mail: gilibertoflor@gmail.com

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Declaración de conflicto de intereses

He recibido honorarios por esta disertación de Sanofi Genzyme

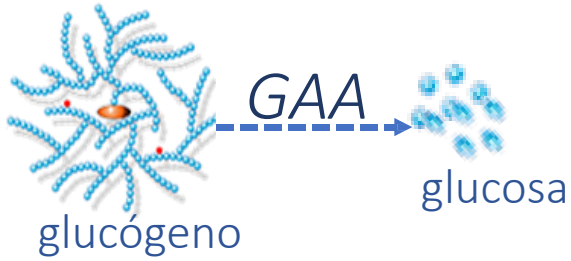
La información contenida en esta presentación está dirigida al profesional de la salud con la intención de brindar información científica de interés y refleja las consideraciones del autor y no las de SANOFI. Cualquier indicación OFF LABEL mencionada será con el único objetivo de intercambio científico y no representa una promoción. SANOFI no recomienda indicaciones que no estén contenidas en la información para prescribir aprobada de sus productos en Colombia.

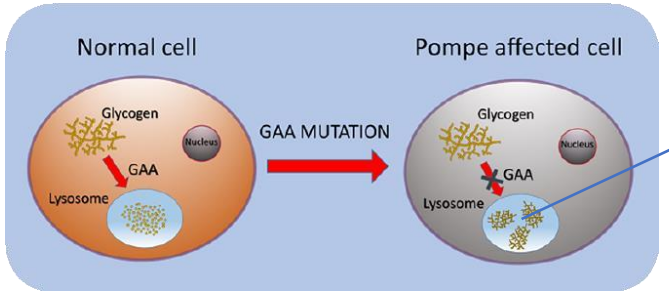
Dra. Flor Giliberto

POMPE

GAA *(α-glucosidasa ácida / α-1,4-glucosidasa)*

Lisosoma





Acumulación de glucógeno: disfunción celular → Manifestaciones multisistémicas

Signos/síntomas asociados a POMPE

Debilidad muscular

Distres respiratorio (LOPD)

CPK ↑

Intolerancia al ejercicio o dolor

Cardiomiopatía hipertrófica (IOPD)

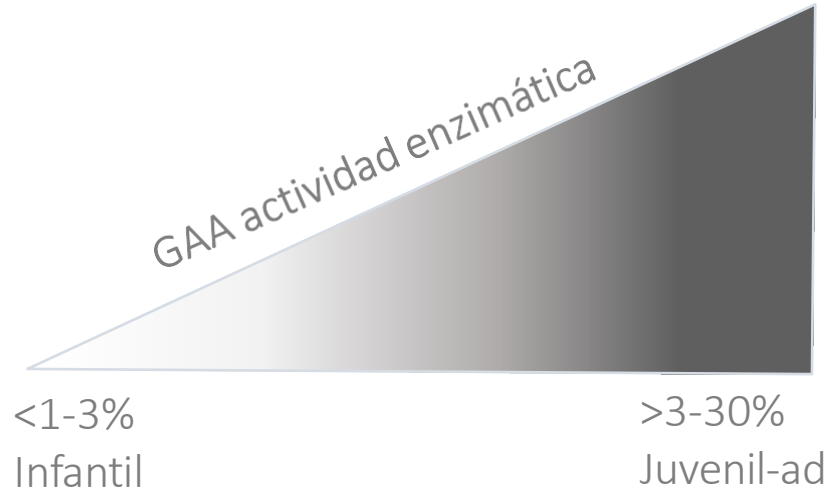
Debilidad muscular severa

Hipotonía

Morbilidad significativa

Muerte prematura en muchos pacientes

Espectro continuo



Güngör et al, Aging 2020; Tchan et al, Neuromuscul Disord. 2020

POMPE: Incidencia

Estimada en (infantil + adulto) 1 : 40.000 a 100.000 recién nacidos

Incidencia: Adulto 1 : 57.000

Infantil 1 : 138.000

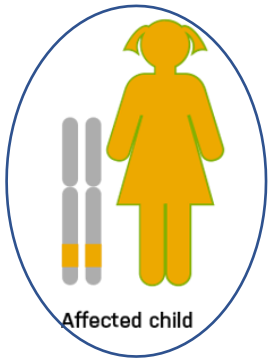
Incidencia varia dependiendo del origen étnico y geográfico

subestimado

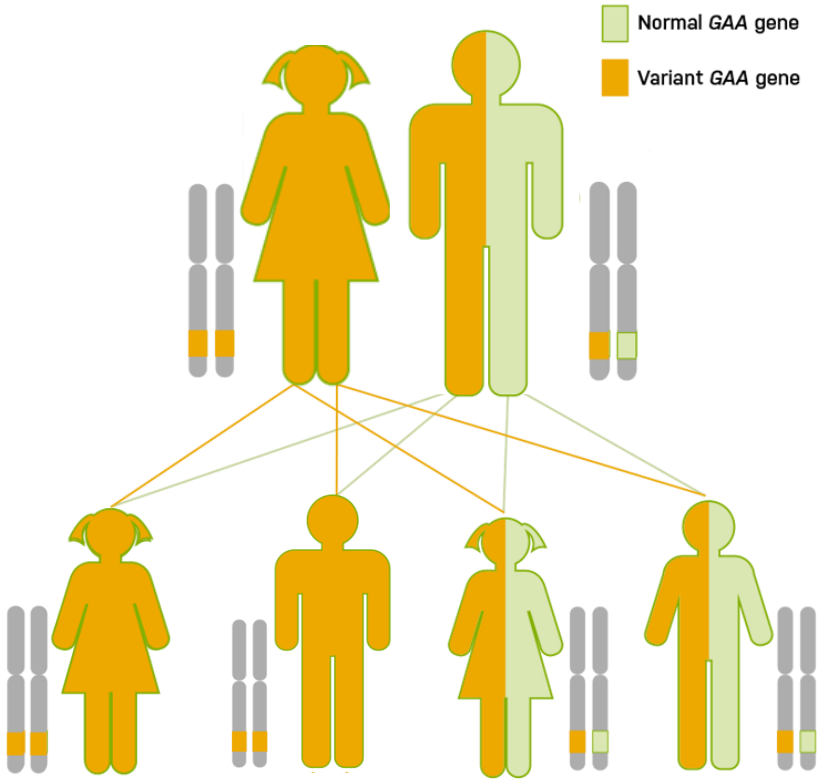
POMPE HERENCIA

Autosómica Recesiva

	N	M
N	NN	NM
M	MN	MM



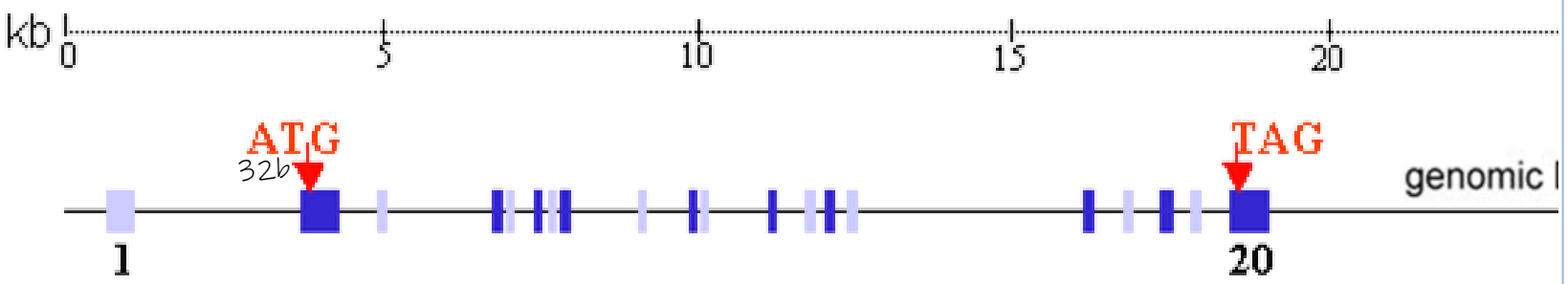
2 variantes asociadas a enfermedad (efecto deficiente o nulo)



50% enfermos-portadores

GAA

(OMIM 606800)*



Expresión

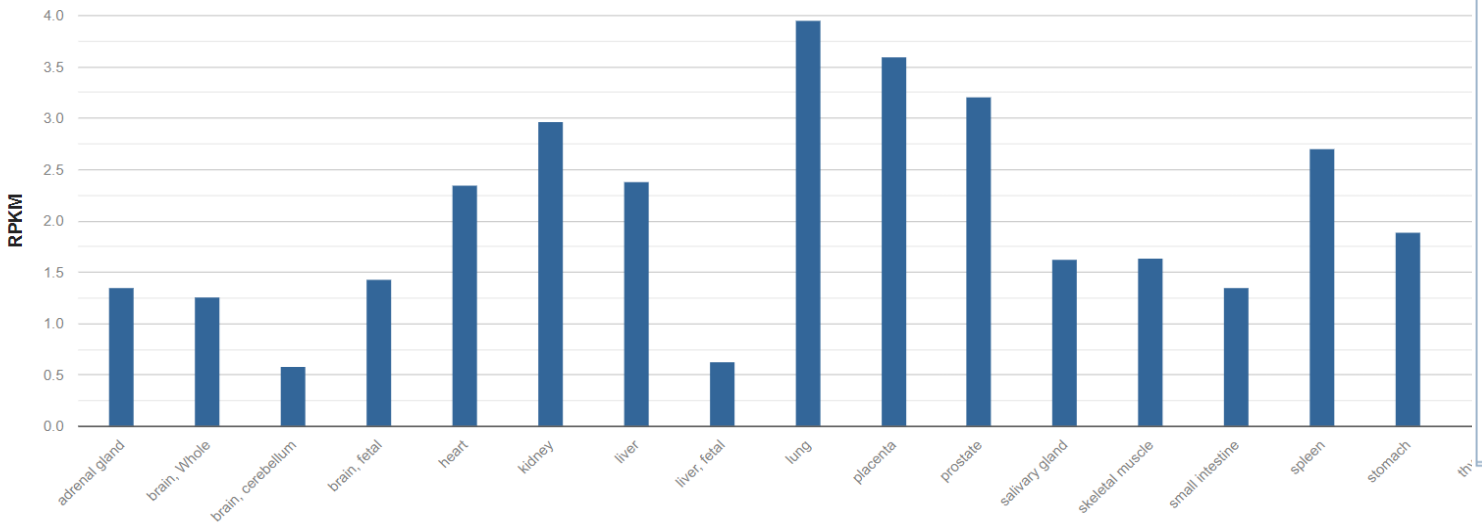
"housekeeping gene"

GAA alpha glucosidase [*Homo sapiens* (human)]

Gene ID: 2548, updated on 3-Jul-2022

RNA sequencing of total RNA from 20 human tissues

- Project title: RNA sequencing of total RNA from 20 human tissues
- Description: RNA sequencing of total RNA from 20 human tissues
- BioProject: PRJNA280600
- Publication: PMID 25970244
- Analysis date: Mon Apr 2 15:22:22 2018



Process

- involved_in cardiac muscle contraction
- involved_in diaphragm contraction
- involved_in glucose metabolic process
- involved_in glycogen catabolic process
- involved_in glycogen catabolic process
- involved_in glycogen catabolic process
- involved_in glycophagy
- involved_in heart morphogenesis
- involved_in locomotory behavior
- involved_in lysosome organization
- involved_in lysosome organization
- involved_in maltose metabolic process
- involved_in muscle cell cellular homeostasis
- involved_in neuromuscular process controlling balance
- involved_in neuromuscular process controlling posture
- involved_in regulation of the force of heart contraction
- involved_in sucrose metabolic process
- involved_in tissue development
- involved_in vacuolar sequestering

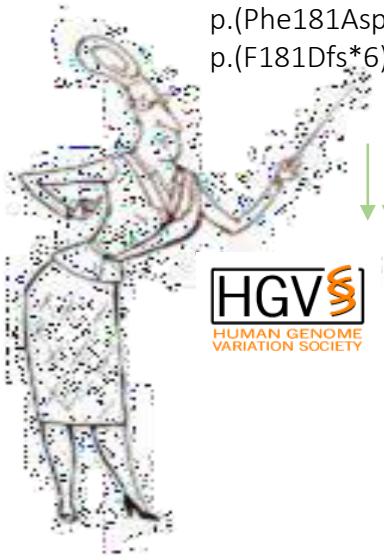
GAA

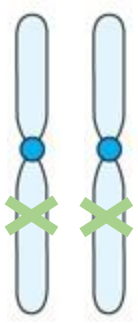
Genotipo → Fenotipo

Gran heterogeneidad clínica, incluso en pacientes con misma variante

20% de las variantes no correlaciona (están presentes en formas infantil y adulto)

más severo — INFANTIL — JUVENIL — ADULTO — menos severo





Frameshift

(c.541_545del)
p.(Phe181AspfsTer6)
p.(Phe181Aspfs*6)
p.(F181Dfs*6)

Missense

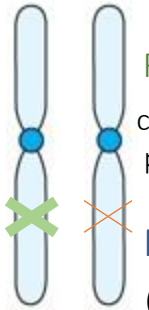
c.1636G>C; p.(Gly546Arg)

Splicing (c.2800-1G>C)

Nonsense

(c.2004C>A)
p.(Tyr668*)

GAA<1%



splicing

(c.-32-13T>G)

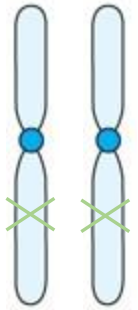
Frameshift

c.766_784del
p.(Tyr256Serfs*6)

Missense

(c.1726G>A)
p.(Gly576Ser)

GAA 1% - 10%



Nonsense

p.(Gln353Ter)

Missense

c.1825T>G
p.(Tyr609Asp)

Splicing

c.692+1G>T

GAA 10% - 20%

HGVS HUMAN GENOME VARIATION SOCIETY

Erasmus MC University Medical Center Rotterdam

(http://www. pompevariantdatabase.nl/)

LOVD Leiden Open Variation Database

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www.pompecenter.nl

Erasmus MC
University Medical Center Rotterdam

Pompe disease GAA variant database

(+ de 900 variantes + de 500 asociadas a enfermedad)

Link to variant	Link to patients	Location	DNA nomenclature	RNA nomenclature	Protein nomenclature	Predicted severity	Phenotype with null allele	CRIM status
Variant info	Patients: 733	intron 1B	c.-32-13T>G	r.[=,-32_546del,-32_486del]	p.[=,0]	Potentially mild	Childhood or Adult	Positive

Patients

Link to patients	Allele 1 DNA	Allele 2 Location	Allele 2 DNA	Allele 2 Phenotype with a null allele	Phenotype of patient	Age of Onset	Gender	Age at analysis	Cardiomyopathy	Liver/ Spleen	Ventilatory support	Respiratory problems	Wheelchair dependency	Mobility problems	(Kypho) Scoliosis	Ptosis	Scapular winging	Cerebral vessels anomalies	No of patients reported	Country/Re
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Adult	35 years	F	48 years	-		-			+ (Walton score: III)		-			1	Portugal
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Adult	38 years	F	47 years	-	-										
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	asymptomatic	asymptomatic	F	68 years	-	-										
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Childhood	13 years	M	13 years												
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Adult	20 years	M	25 years											1	Italy
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Adult (1)/ Childhood (1)	48 years/6 years		49 years/8 years	-/-		-/-		-/-	-/-	-/-				2	Germany
PubMed	c.-32-13T>G	intron 1B	c.-32-13T>G	Childhood or Adult	Adult (2)	49 years/40 years	M/M	51 years/42 years	-/slight left ventricular hypertrophy		-/-	-/-	-/-	-/-			-/-		2	Italy

A genetic modifier of symptom onset in Pompe disease

Atze J. Bergsma [a,b,c], Stijn L.M. in 't Groen [a,b,c], Jan J.A. van den Dorpel [a,c], Hannerieke J.M.P. van den Hout [a,c], Nadine A.M.E. van der Beek [a,c], Benedikt Schoser [d], Antonio Toscano [e], Olimpia Musumeci [e], Bruno Bembi [f], Andrea Dardis [f], Amelia Morrone [g], Albina Tummolo [h], Elisabetta Pasquini [i,†], Ans T. van der Ploeg [a,c], W.W.M. Pim Pijnappel [a,b,c,*]

c.510C>T *GAA* variant as a genetic modifier of symptom onset and splicing in Pompe disease

Peruzzo et al. 2019

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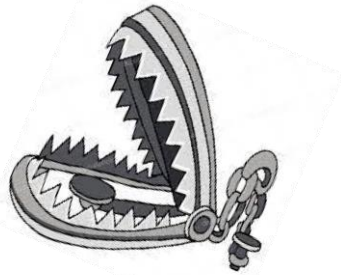


DIAGNÓSTICO DE **POMPE**

Confirmación Genética

Confirmación enzimática

Confirmación Genética + Enzimática

ALGORITMO DIAGNÓSTICO

Sin Clínica

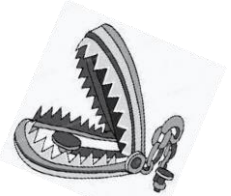
Clínica compatible con POMPE

Hallazgo incidental:

-NBS screening neonatal (Ez deficiente)
-PGD (Dx prenatal genético)
-Dx x familiar con POMPE

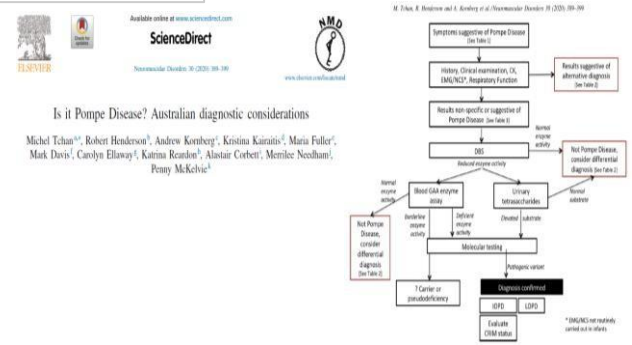
Estudio genético
2 variantes patogénicas en trans

Falta la 2da variante VUS

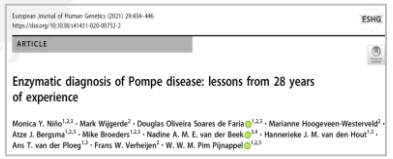


Alelos de pseudodeficiencia!

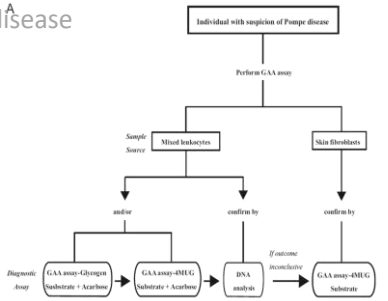
Actividad Ez (GAA)
DBS x2



Actividad Ez (GAA)
Leucocitos-Fibroblastos-Músculo



Flow chart for diagnosis of Pompe disease



Más temprano

Dx TEMPRANO!
SEGUIMIENTO PREVENTIVO
ACCIONAR MÉDICO

Mejor

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Confirmar el diagnóstico clínico diferencial de POMPE

Estándares de cuidado

Tchan, Neuromuscul Disord. 2020

Asesoramiento Genético

Diagnóstico temprano

Seguimiento preventivo de pacientes asintomáticos

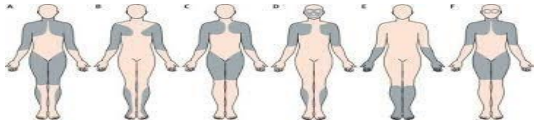
Prevenir/disminuir **daño de órganos**

Tratamiento de reemplazo enzimático

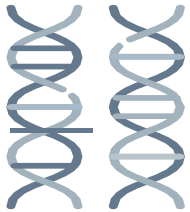
DIAGNÓSTICO MOLECULAR

¿Por donde comienzo a buscar?

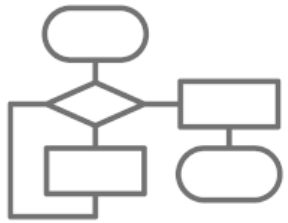
Diagnóstico clínico presuntivo orientador



GENES involucrados en la fisiopatología

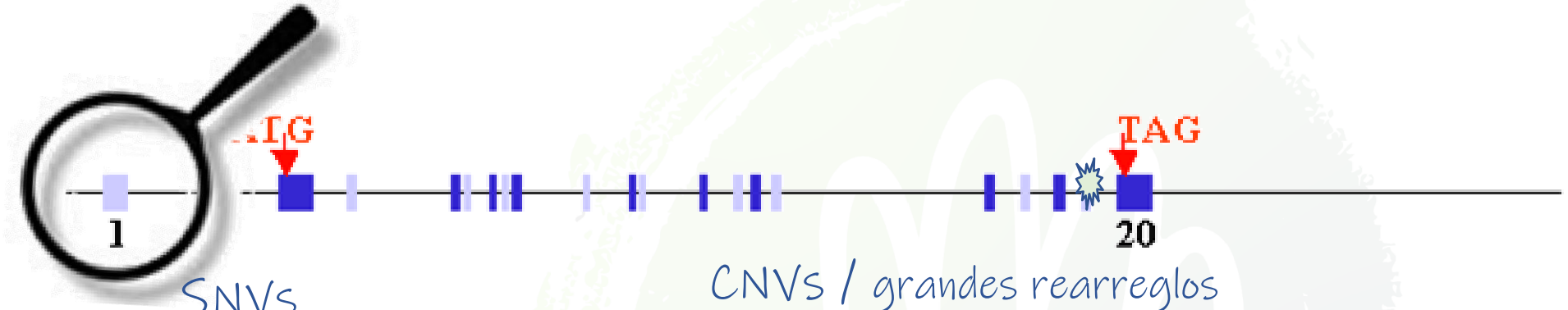


Tipo de alteraciones tengo que analizar



ALGORITMO

GAA alteraciones: Diagnóstico Molecular



SNVs

missense
nonsense
splice-site
indels

~95,8%

Secuenciación (NGS/Sanger)

CNVs / grandes reagrreglos

Rearreglos complejos

deleciones inserciones

duplicaciones

~4.2%

MLPA / CGH ARRAY /qPCR

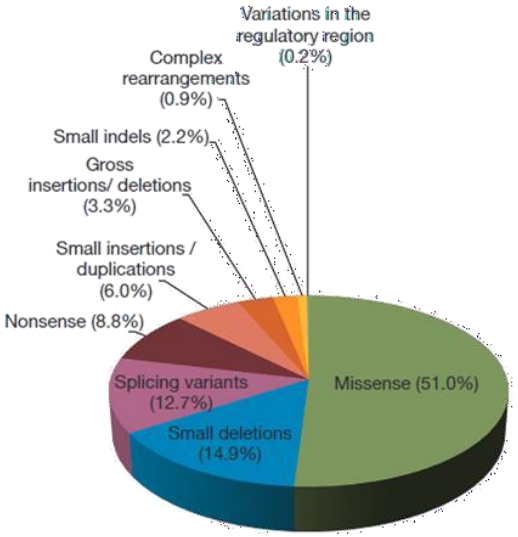


Figure 1 Frequency of GAA mutant alleles reported in the HGMD-http://www.hgmd.cf.ac.uk/ac/ classified by mutation type. GAA, acid alpha-glucosidase.

Peruzzo et al, 2019

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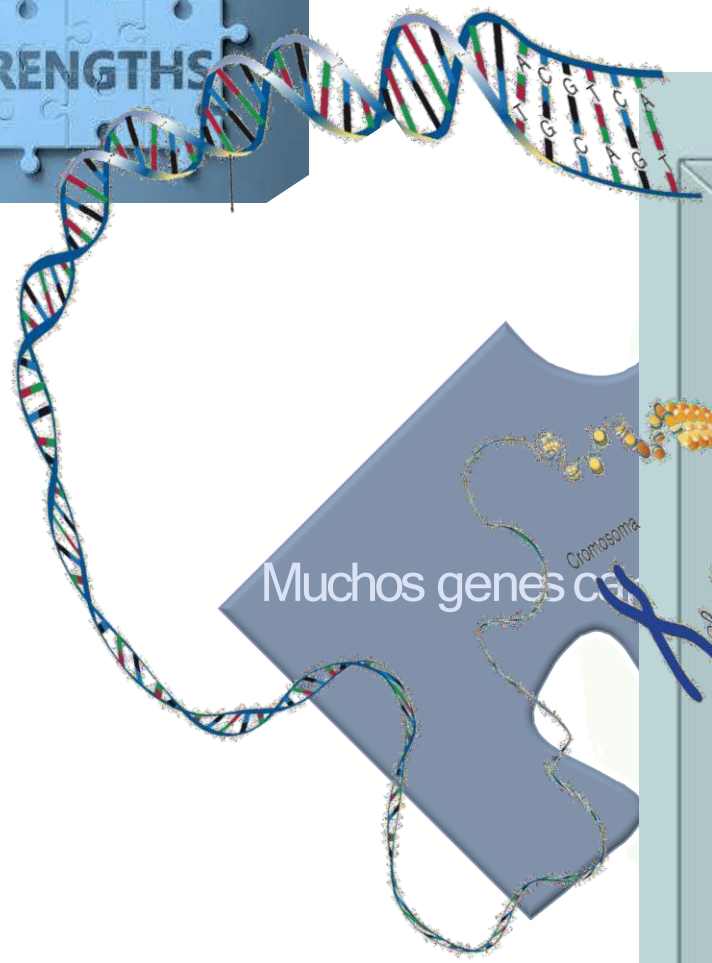
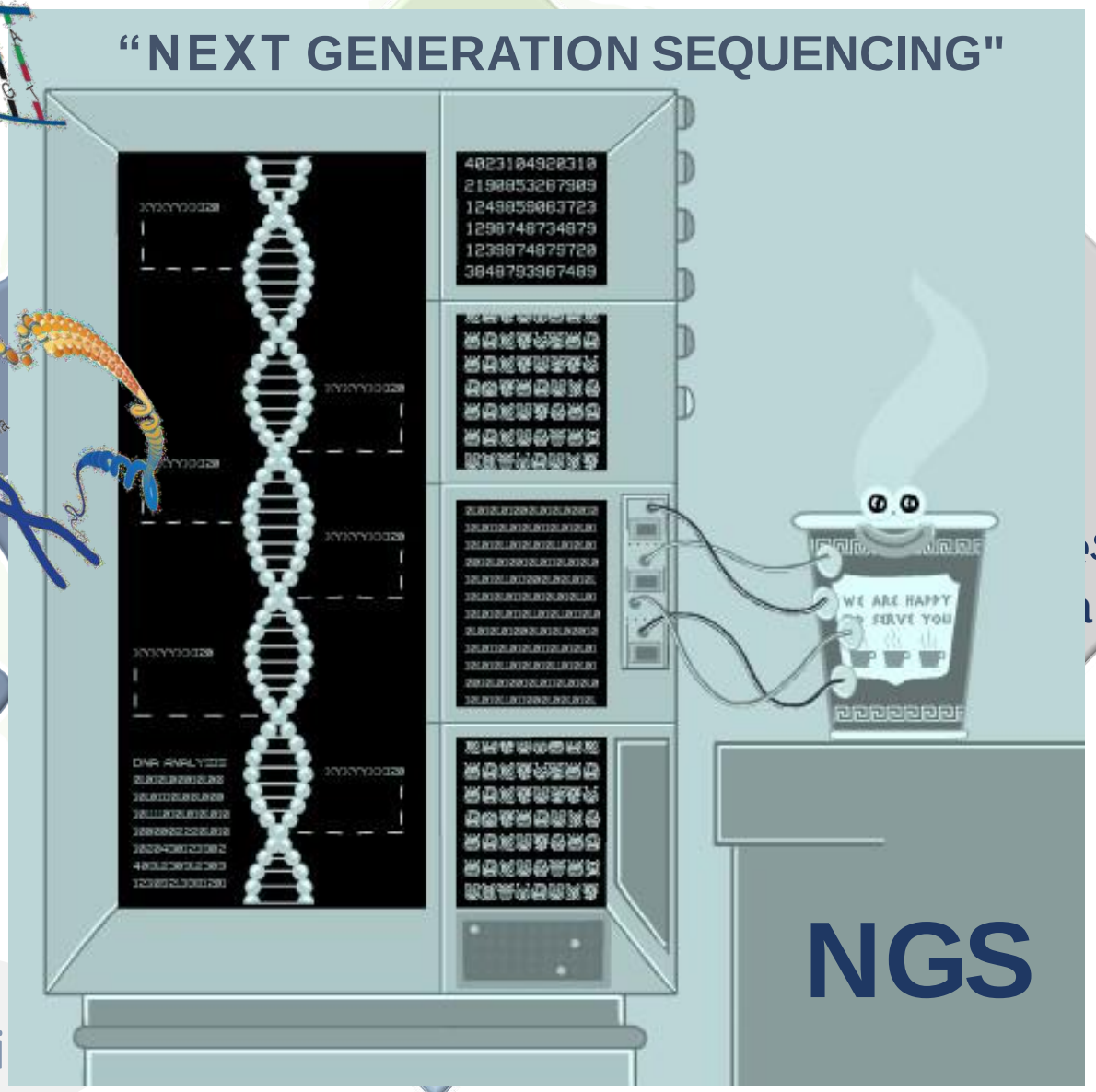
STRENGTHS



Muchos genes c

“NEXT GENERATION SEQUENCING"



NGS

Primer-línea de estud¡

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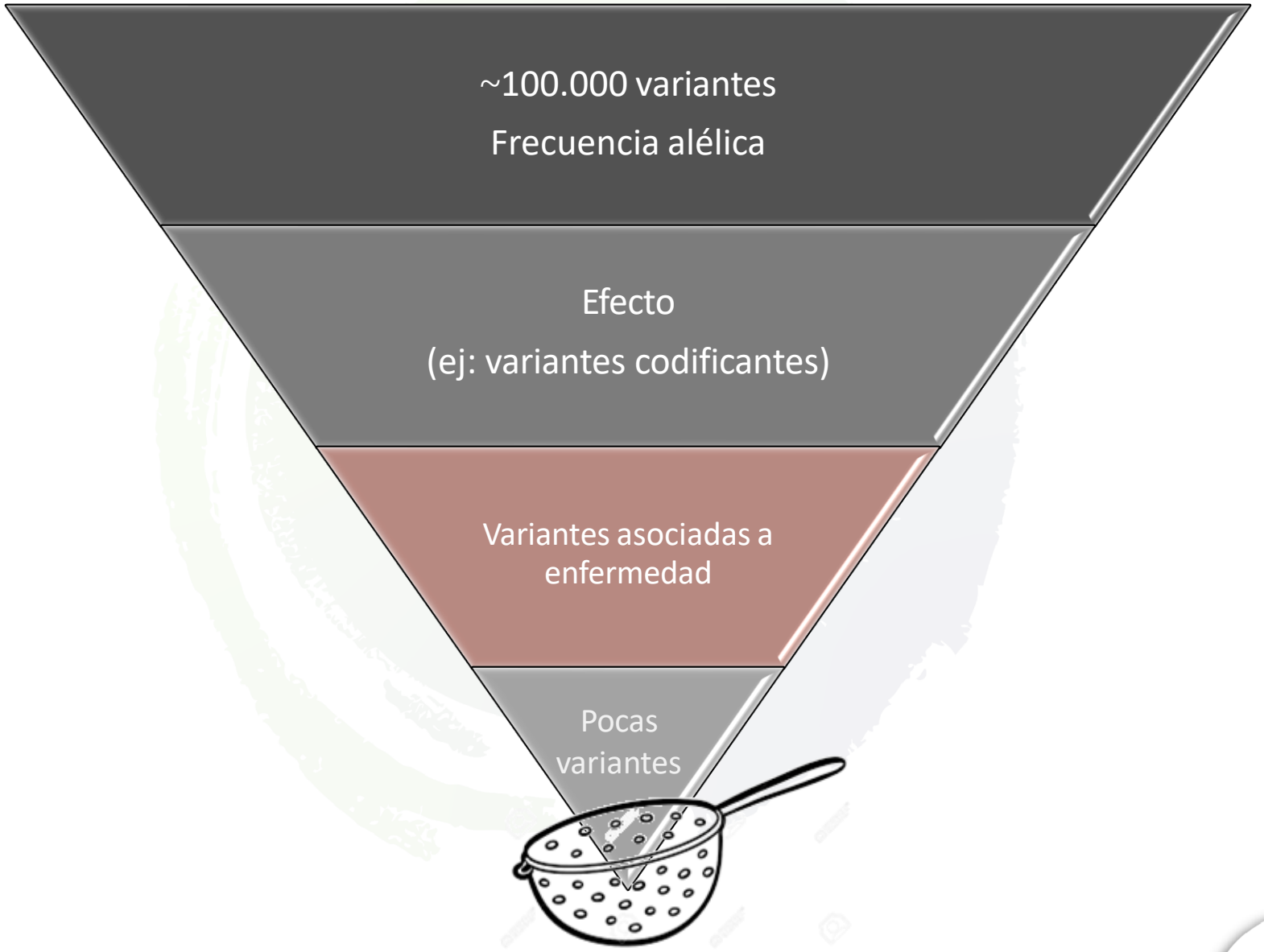
Exoma / Panel
?
?
?
?
?
?
?
?
?

Filtrado de variantes



Variant assessment checklist

Gene-level information

- ☑ ☐ Confirm gene is implicated in disease with sufficient evidence, including human genetic data and functional data
- ☐ Determine inheritance pattern, age-of-onset, penetrance and prevalence for each gene-disease association, if possible
- ☑ ☐ Determine types of disease-associated variants in gene (gain-of-function, loss-of function, etc.)

Variant validation

- ☐ Review raw sequence data to confirm the variant call
- ☑ ☐ Determine zygosity of the variant
- ☐ Associate genome build, genomic coordinate, and reference transcript to the variant
- ☑ ☐ Confirm variant nomenclature

Genetic data

- ☐ Determine frequency of variant in large population studies, parsed by race
- ☑ ☐ Determine if population frequency is consistent with disease inheritance, age-of-onset, penetrance and prevalence
- ☐ Evaluate whether variant segregates with disease in affected family members
- ☐ If disease is inherited in a recessive manner, determine if the variant is found *in trans* with a pathogenic variant
- ☑ ☐ If applicable, determine if there is a statistically significant difference in variant frequency between cases and controls

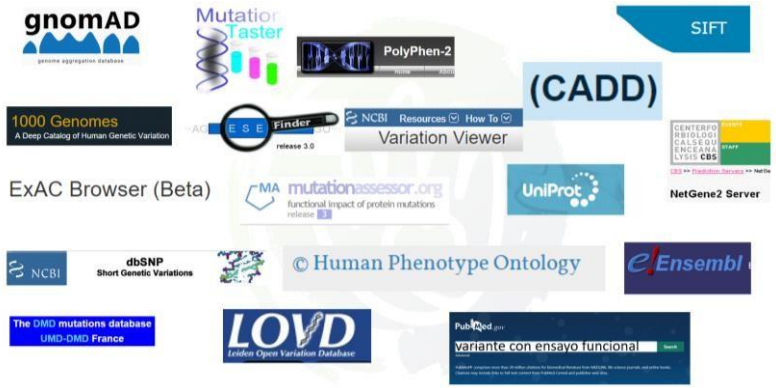
Functional data

- ☑ ☐ Evaluate available *in vivo* functional data
  - ☐ Confirm type of animal model is relevant for human disease
- ☐ Evaluate available *in vitro* functional data
  - ☑ ☐ Confirm assays used reflect disease-associated cellular mechanisms

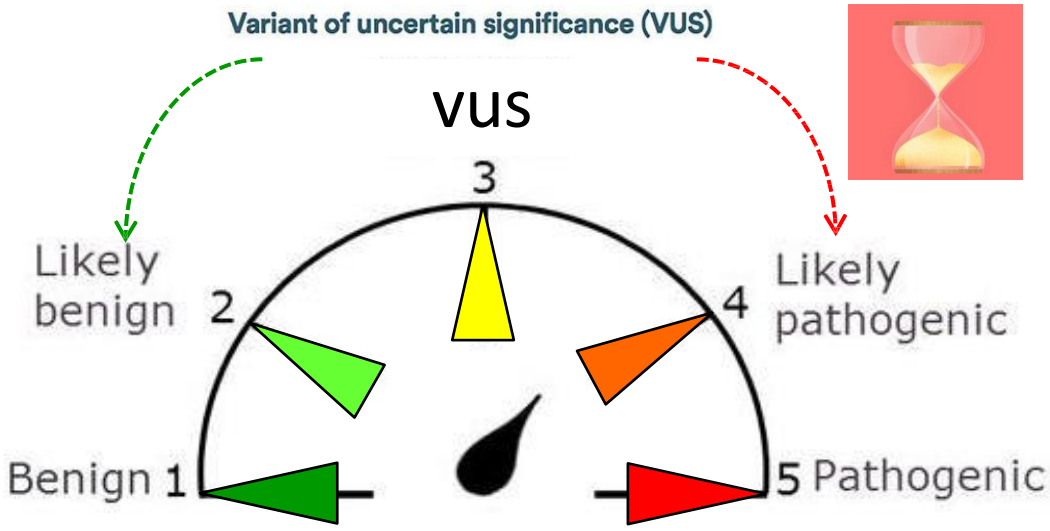
Computational data

- ☐ Evaluate nucleotide alignment data and assess evolutionary conservation (for all variants)
- ☑ ☐ Evaluate amino acid alignment data and assess evolutionary conservation (for missense variants)
  - ☐ Eliminate any poor species alignments
- ☑ ☐ Determine if computational tools predict an effect on protein structure or splicing (for all variants)

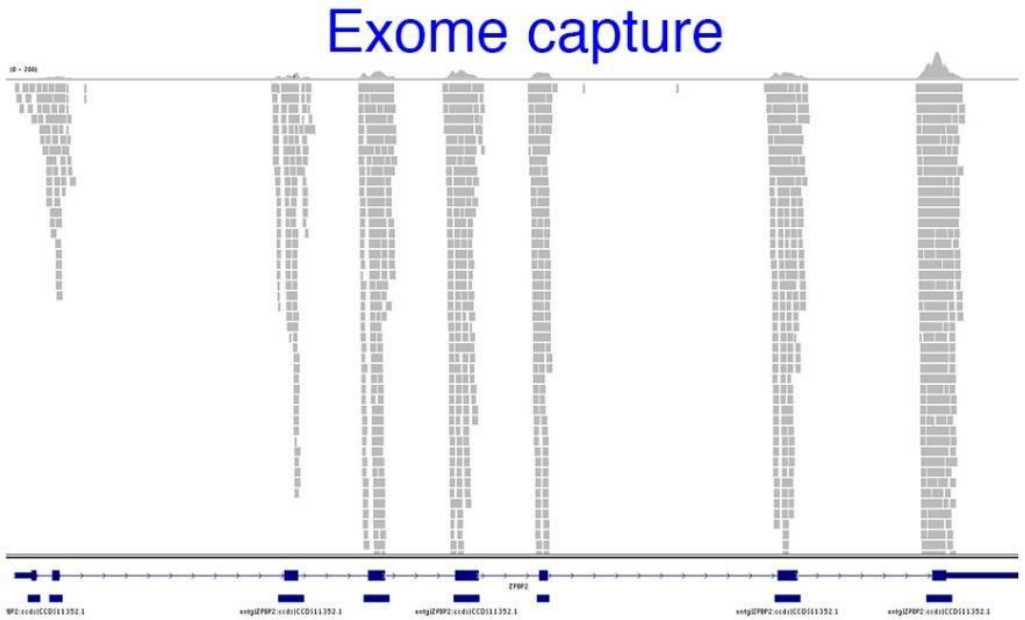
Genet Med. 2015; 17(5):405-24. doi: 10.1038/gim



CLASIFICACIÓN DE LAS VARIANTES (ACMG interpretación)

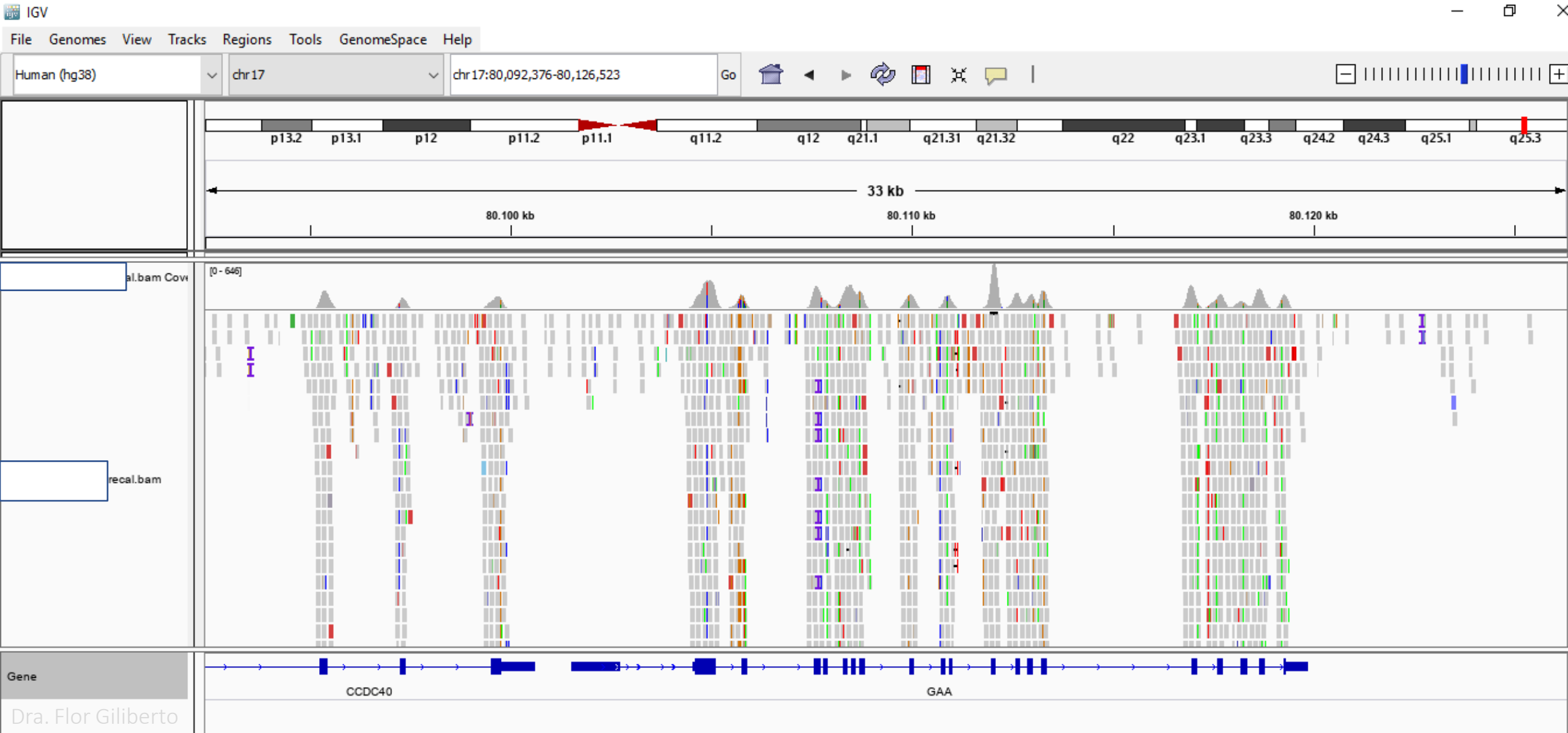


NGS Ilustración

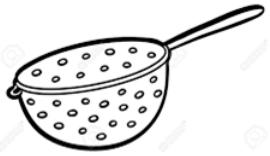


(*GAA*)

Case NGS (*GAA*)


IGV
File
Genomes
View
Tracks
Regions
Tools
GenomeSpace
Help
Human (hg38)
chr17
chr17:80,092,376-80,126,523
Go
p13.2
p13.1
p12
p11.2
p11.1
q11.2
q12
q21.1
q21.31
q21.32
q22
q23.1
q23.3
q24.2
q24.3
q25.1
q25.3
33 kb
80.100 kb
80.110 kb
80.120 kb
[0 - 646]
Gene
CCDC40
GAA

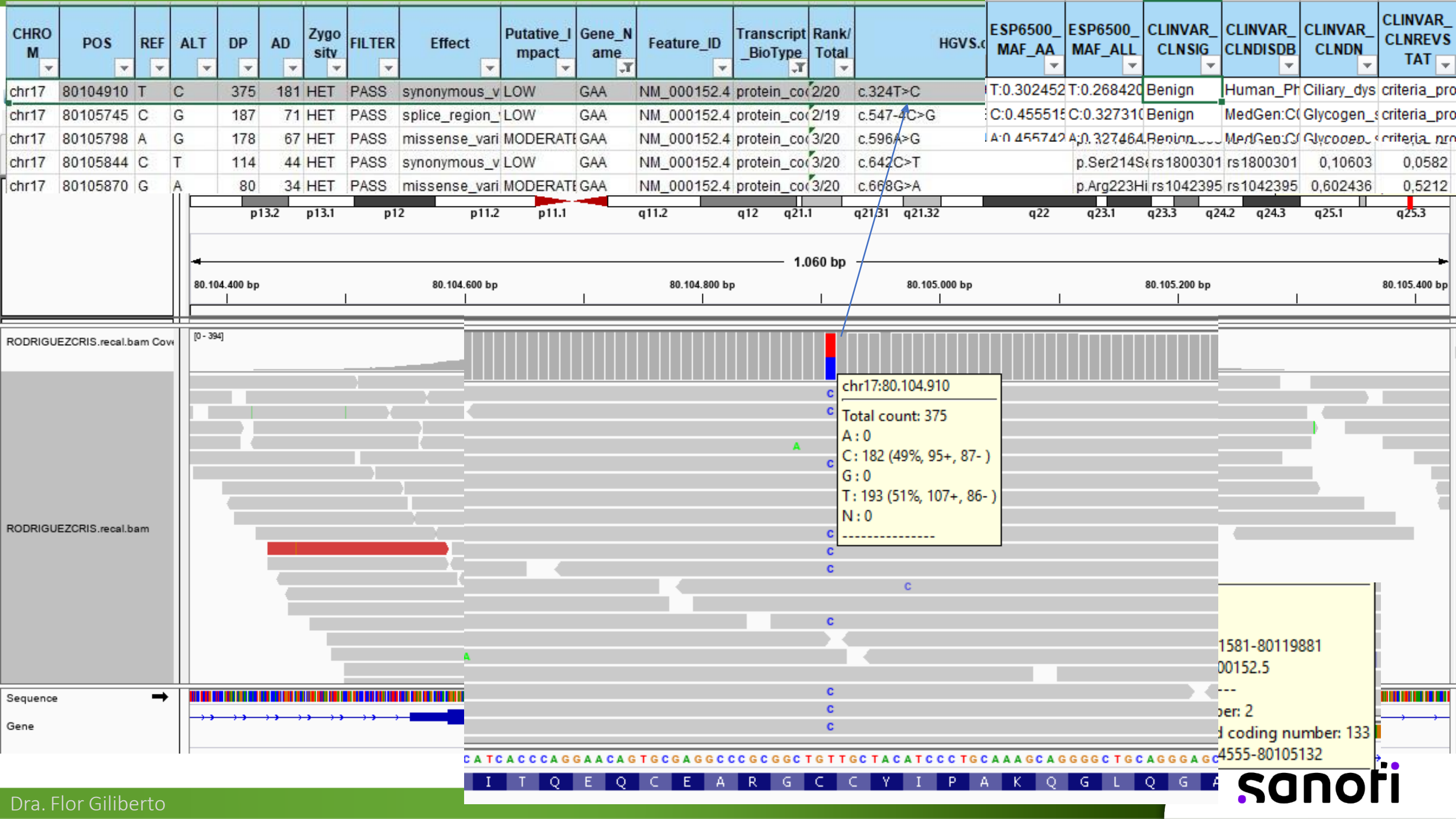
Caso NGS (GAA)



1	CHROM	POS	REF	ALT	DP	AD	Zygosit	FILTER	Effect	Putative_Impact	Gene_Name	Feature_ID	Transcript_BioType	Rank/Tot	HGVS.c	HGVS.p	dbSNP138_ID	dbSNP151_ID	p3_1000G_AF	p3_1000G_AFR_A	p3_1000G_AMR_A	p3_1000G_EAS_A	p3_1000G_EUR_A	p3_1000G_SAS_A
94359	chr17	8E+07	T	C	375	181	HET	PASS	synonymous_	LOW	GAA	NM_000152.4	protein_co	2/20	c.324T>C	p.Cys108C	rs1800300	rs1800300	0,71446	0,7186	0,562	0,6518	0,7634	0,8313
94363	chr17	8E+07	C	G	187	71	HET	PASS	splice_region_	LOW	GAA	NM_000152.4	protein_co	2/19	c.547-4C>G	.	rs3816256	rs3816256	0,60284	0,5212	0,5418	0,5437	0,7535	0,6626
94364	chr17	8E+07	A	G	178	67	HET	PASS	missense_vari	MODERAT	GAA	NM_000152.4	protein_co	3/20	c.596A>G	p.His199A	rs1042393	rs1042393	0,60084	0,5212	0,5418	0,5437	0,7535	0,6524
94365	chr17	8E+07	C	T	114	44	HET	PASS	synonymous_	LOW	GAA	NM_000152.4	protein_co	3/20	c.642C>T	p.Ser214S	rs1800301	rs1800301	0,10603	0,0582	0,1138	0,002	0,2276	0,1472
94366	chr17	8E+07	G	A	80	34	HET	PASS	missense_vari	MODERAT	GAA	NM_000152.4	protein_co	3/20	c.668G>A	p.Arg223H	rs1042395	rs1042395	0,60244	0,5212	0,5418	0,5437	0,7535	0,6605
94367	chr17	8E+07	A	AGCA	196	104	HET	PASS	splice_region_	LOW	GAA	NM_000152.4	protein_co	4/19	c.858+7_858+8	.	rs3071247	rs3071247	.	.	.	.	.	.
94368	chr17	8E+07	T	C	159	89	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	4/19	c.858+30T>C	.	rs2304845	rs2304845	0,60104	0,5174	0,5403	0,5437	0,7515	0,6616
94369	chr17	8E+07	G	A	74	40	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	5/19	c.955+12G>A	.	rs2252455	rs2252455	0,60443	0,5189	0,5418	0,5446	0,7545	0,6718
94370	chr17	8E+07	G	A	57	24	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	5/19	c.956-107G>A	.	rs2241888	rs2241888	0,71246	0,7126	0,5634	0,6498	0,7614	0,8323
94371	chr17	8E+07	C	T	74	31	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	5/19	c.956-84C>T	.	rs2241887	rs2241887	0,60363	0,5174	0,5418	0,5437	0,7515	0,6738
94372	chr17	8E+07	G	A	243	125	HET	PASS	synonymous_	LOW	GAA	NM_000152.4	protein_co	8/20	c.1203G>A	p.Gln401G	rs1800304	rs1800304	0,60284	0,5166	0,5418	0,5427	0,7515	0,6718
94373	chr17	8E+07	A	G	187	90	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	8/19	c.1327-18A>G	.	rs2278619	rs2278619	0,71166	0,7118	0,5634	0,6488	0,7604	0,8313
94374	chr17	8E+07	G	C	22	12	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	9/19	c.1438-19G>C	.	rs2304844	rs2304844	0,60304	0,5182	0,5432	0,5417	0,7515	0,6708
94375	chr17	8E+07	C	A	185	107	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	10/19	c.1551+49C>A	.	rs2304843	rs2304843	0,59924	0,5113	0,5432	0,5427	0,7515	0,6595
94376	chr17	8E+07	GGC	G	6	4	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	11/19	c.1636+117_163	.	.	rs1491342	.	.	.	.	.	.
94377	chr17	8E+07	G	T	5	4	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	11/19	c.1636+119G>T	.	.	rs1050581	.	.	.	.	.	.
94378	chr17	8E+07	C	T	25	7	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	12/19	c.1754+144C>T	.	rs2304838	rs2304838	0,52017	0,4894	0,5086	0,3036	0,7137	0,5941
94379	chr17	8E+07	A	G	116	53	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	14/19	c.2040+20A>G	.	rs2304836	rs2304836	0,71506	0,7179	0,5677	0,6508	0,7634	0,8323
94380	chr17	8E+07	G	A	77	44	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	14/19	c.2041-64G>A	.	rs2304833	rs2304833	0,22804	0,1566	0,1671	0,1964	0,3181	0,3078
94381	chr17	8E+07	A	G	236	120	HET	PASS	synonymous_	LOW	GAA	NM_000152.4	protein_co	15/20	c.2133A>G	p.Thr711T	rs1800310	rs1800310	0,24161	0,0877	0,1643	0,3819	0,3052	0,2945
94382	chr17	8E+07	G	A	75	40	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	16/19	c.2331+20G>A	.	rs2304832	rs2304832	0,78934	0,9834	0,5965	0,6498	0,7654	0,8323
94383	chr17	8E+07	A	T	39	20	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	16/19	c.2332-198A>T	.	rs2304830	rs2304830	0,71426	0,7148	0,5706	0,6498	0,7624	0,8323
94384	chr17	8E+07	G	A	140	77	HET	PASS	missense_vari	MODERAT	GAA	NM_000152.4	protein_co	17/20	c.2338G>A	p.Val780I	rs1126690	rs1126690	0,71186	0,7156	0,5519	0,6498	0,7624	0,8323
94385	chr17	8E+07	G	A	86	38	HET	PASS	synonymous_	LOW	GAA	NM_000152.4	protein_co	18/20	c.2553G>A	p.Gly851G	rs1042397	rs1042397	0,50939	0,5439	0,4942	0,2887	0,663	0,5429
94386	chr17	8E+07	G	A	121	69	HET	PASS	intron_variant	MODIFIER	GAA	NM_000152.4	protein_co	19/19	c.2800-60G>A	.	rs5566248	rs5566248	0,01458	0,003	0,0735	0,001	0,008	0,0092
113719																								

SNP_Indel_ANNO

Listo Se encontraron 25 de 113717 registros Promedio: 1844,1875 Recuento: 234 Suma: 88521


CHROM
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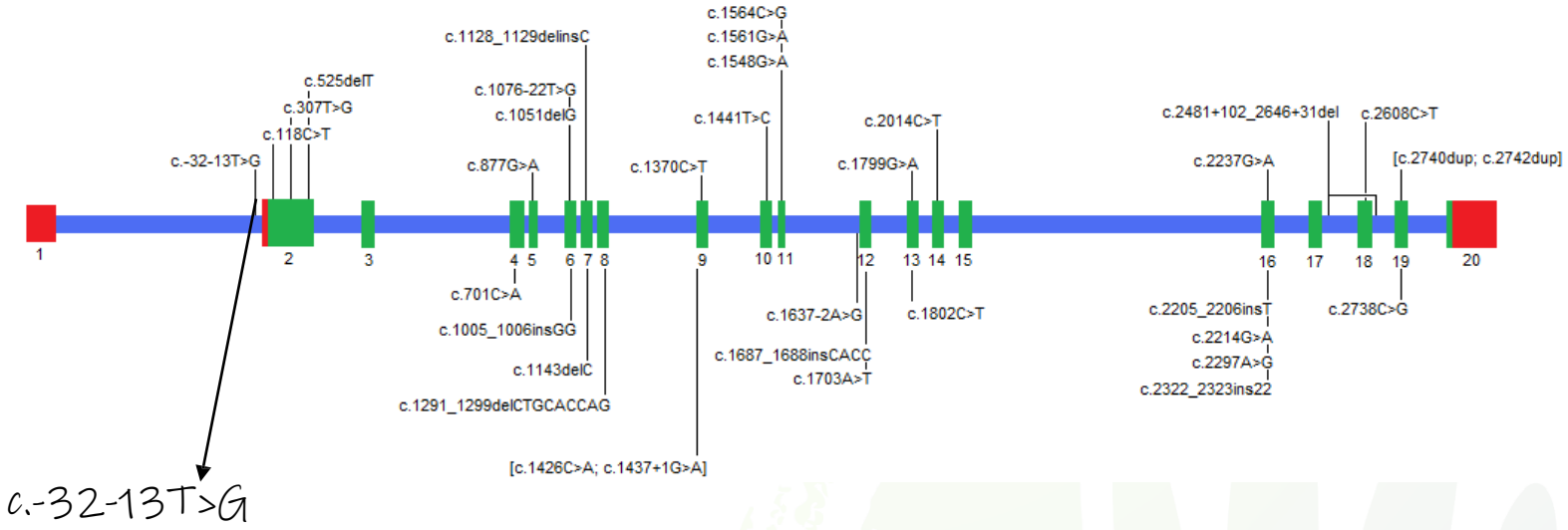
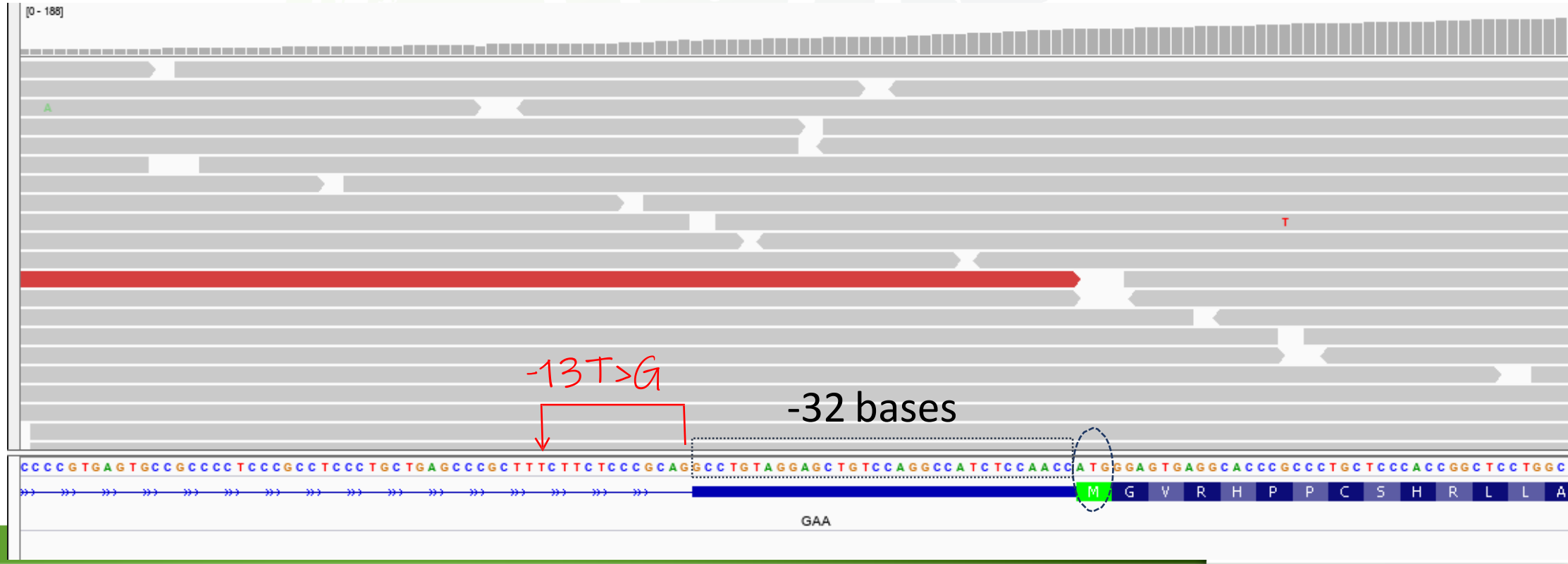
FILTER
Effect
Putative_Impact
Gene_Name
Feature_ID
Transcript_BioType
Rank/Total
HGVS.c
ESP6500_MAF_AA
ESP6500_MAF_ALL
CLINVAR_CLNSIG
CLINVAR_CLNDISDB
CLINVAR_CLNDN
CLINVAR_CLNREVSTAT
chr17 80104910 T C 375 181 HET PASS synonymous_v LOW GAA NM_000152.4 protein_co 2/20 c.324T>C T:0.302452 T:0.26842(Benign Human_Ph Ciliary_dys criteria_pro
chr17 80105745 C G 187 71 HET PASS splice_region_ LOW GAA NM_000152.4 protein_co 2/19 c.547-4C>G C:0.455515 C:0.32731(Benign MedGen:C(Glycogen_s criteria_pro
chr17 80105798 A G 178 67 HET PASS missense_vari MODERATE GAA NM_000152.4 protein_co 3/20 c.596A>G A:0.455742 A:0.327464 Benign MedGen:C(Glycogen_s criteria_pro
chr17 80105844 C T 114 44 HET PASS synonymous_v LOW GAA NM_000152.4 protein_co 3/20 c.642C>T p.Ser214S(rs1800301 rs1800301 0,10603 0,0582
chr17 80105870 G A 80 34 HET PASS missense_vari MODERATE GAA NM_000152.4 protein_co 3/20 c.668G>A p.Arg223Hi rs1042395 rs1042395 0,602436 0,5212
p13.2 p13.1 p12 p11.2 p11.1 q11.2 q12 q21.1 q21.31 q21.32 q22 q23.1 q23.3 q24.2 q24.3 q25.1 q25.3
1.060 bp
80.104.400 bp
80.104.600 bp
80.104.800 bp
80.105.000 bp
80.105.200 bp
80.105.400 bp
RODRIGUEZCRIS.recal.bam Cov
[0 - 394]
RODRIGUEZCRIS.recal.bam
chr17:80.104.910
Total count: 375
A : 0
C : 182 (49%, 95+, 87-)
G : 0
T : 193 (51%, 107+, 86-)
N : 0
Sequence
Gene
I T Q E Q C E A R G C C Y I P A K Q G L Q G


c.-32-13T>G
c.525delT
c.307T>G
c.118C>T
c.1128_1129delinsC
c.1076-22T>G
c.1051delG
c.877G>A
c.1564C>G
c.1561G>A
c.1548G>A
c.1441T>C
c.1370C>T
c.2014C>T
c.1799G>A
c.2481+102_2646+31del
c.2608C>T
c.2237G>A
[c.2740dup; c.2742dup]
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
c.701C>A
c.1005_1006insGG
c.1143delC
c.1291_1299delCTGCACCAG
[c.1426C>A; c.1437+1G>A]
c.1637-2A>G
c.1687_1688insCACC
c.1703A>T
c.1802C>T
c.2205_2206insT
c.2214G>A
c.2297A>G
c.2322_2323ins22
c.2738C>G
c.-32-13T>G


[0 - 188]
-13T>G
-32 bases
GAA

MRC Holland

Products Technology About Us News & Events Support

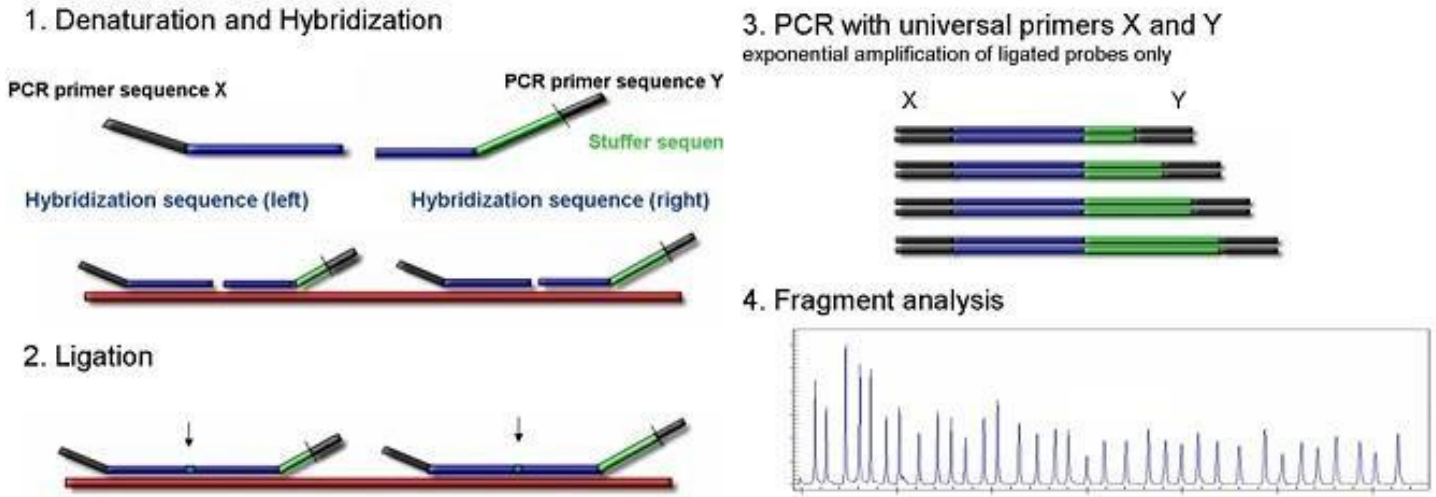
SALSA MLPA Probemix P453 GAA

Pompe disease

Region: 17q25.3

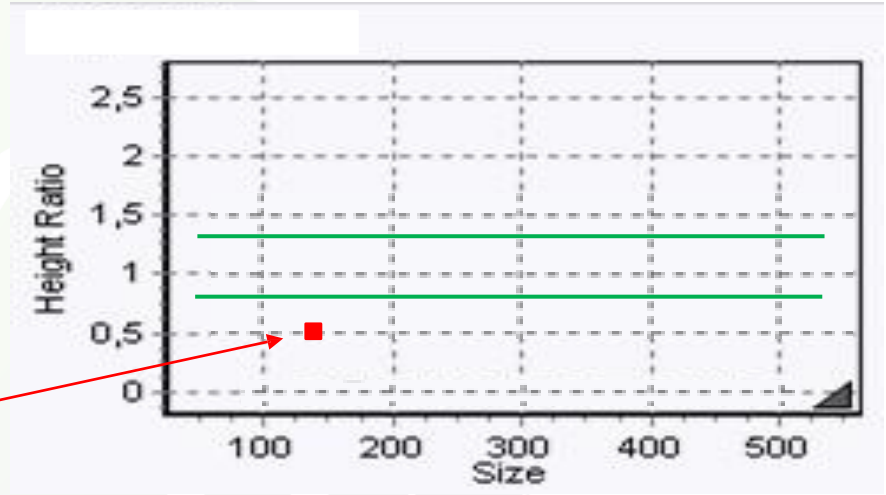
MLPA

MLPA

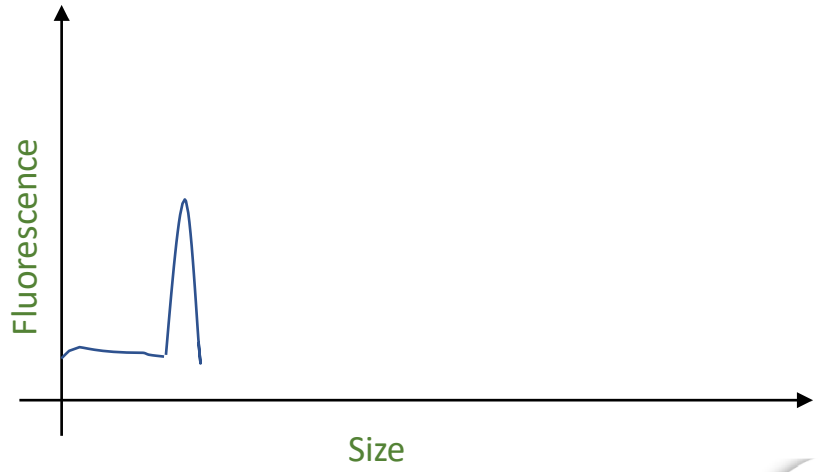


sanofi

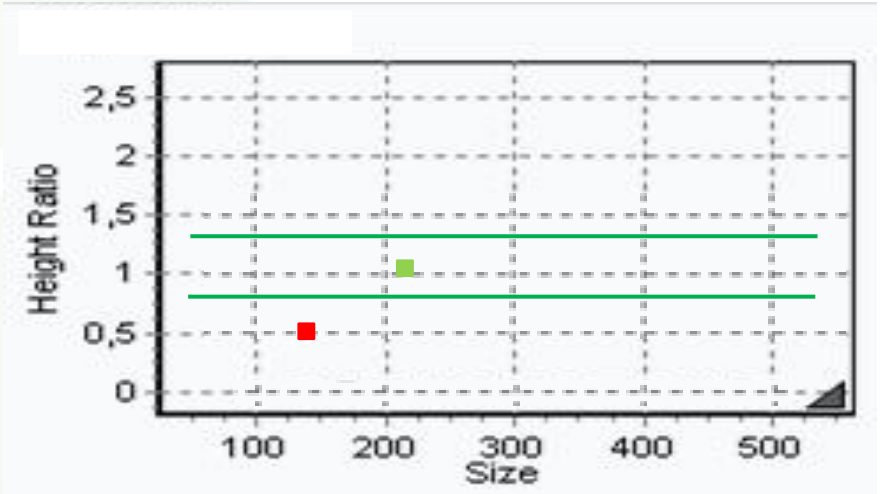
MLPA



Deleción




-
+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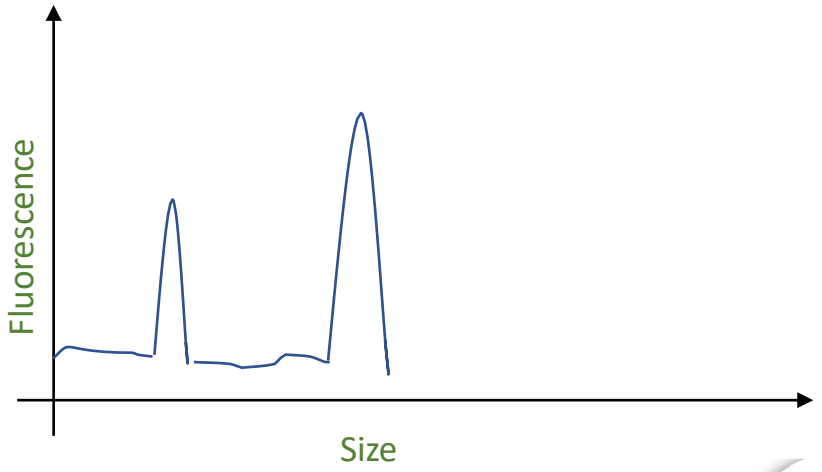
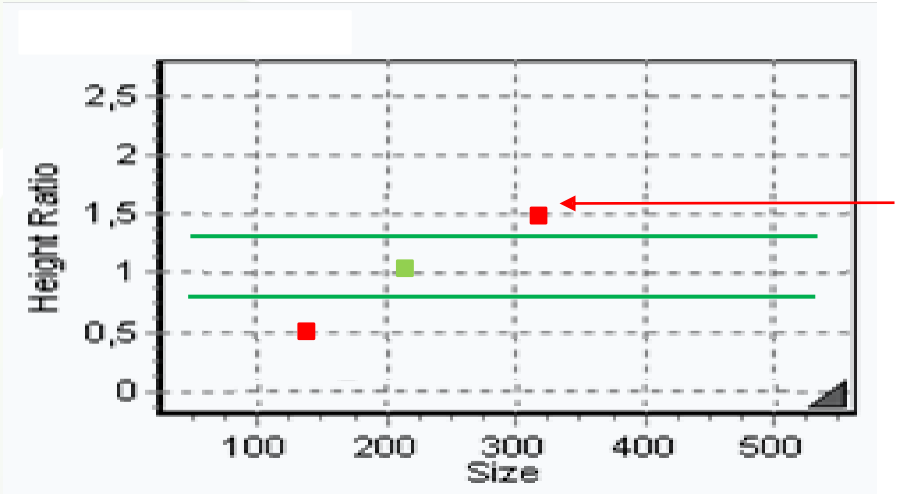
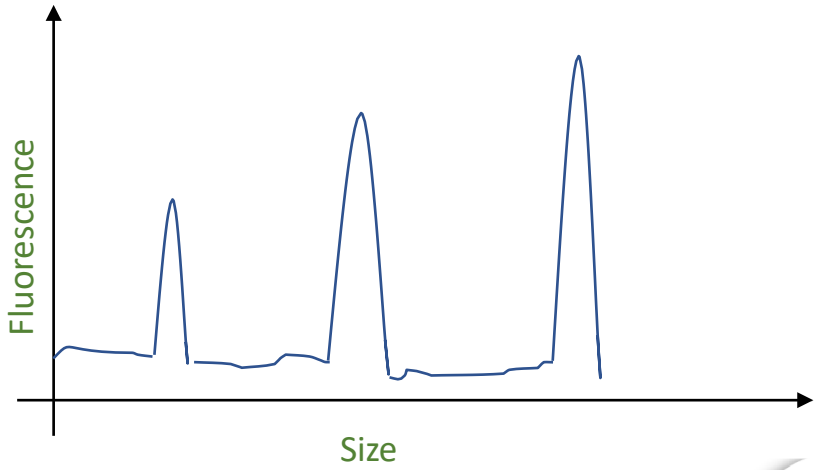
1,5
1
0,5
0
100
200
300
400
500
Size


Fluorescence
Size



Duplicación



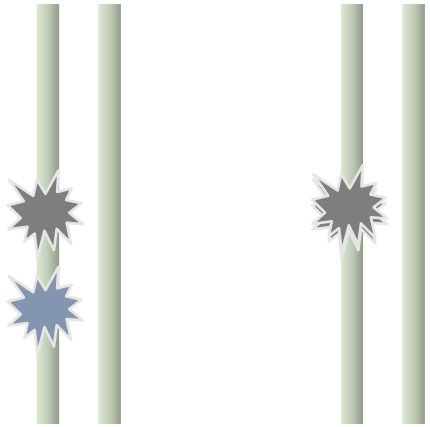
CASOS

CASO 1

Paciente
Clínica compatible con POMPE

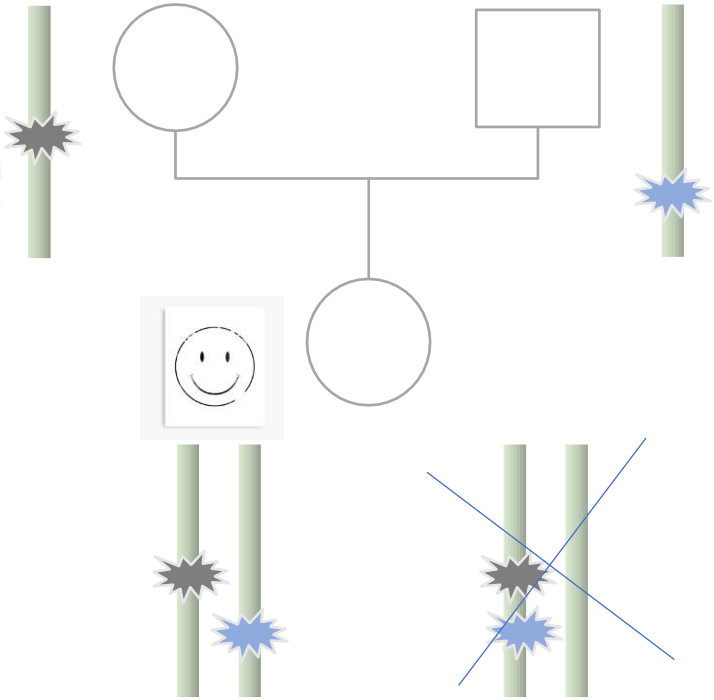
Estudio genético
2 variantes
Patogénicas

Qué hago?



Cómo lo hago?

Segregación



CASO 2

Paciente
Clínica compatible con POMPE

Actividad Ez ↓ DBS

o

Estudio genético
1 variantes Patogénicas
O 2 VUS

Qué hago?
Repito

Actividad Ez DBS Normal → **Descarto POMPE**

Actividad Ez ↓ DBS → **Qué hago?**

Actividad Ez fibroblastos Normal Leucos, músculo → **Descarto POMPE**

Actividad Ez fibroblastos ↓ Leucos, músculo → **Confirmo POMPE**

Qué hago?

Estudio genético
2 variantes Patogénicas → **Confirmo POMPE**

CASO 3

Más que un caso una obsesión

Más que un caso una obsesión

OPEN **Whole-exome sequencing of the mummified remains of Cangrande della Scala (1291–1329 CE) indicates the first known case of late-onset Pompe disease** (2021).

- Nobleman died almost 700 years ago
- First exome analysis of a mummy genome
- Likely represents the earliest known case of this autosomal recessive metabolic disorder

Cangrande della Scala (1291–1329 CE)
Lord of Verona

exhumed in 2004

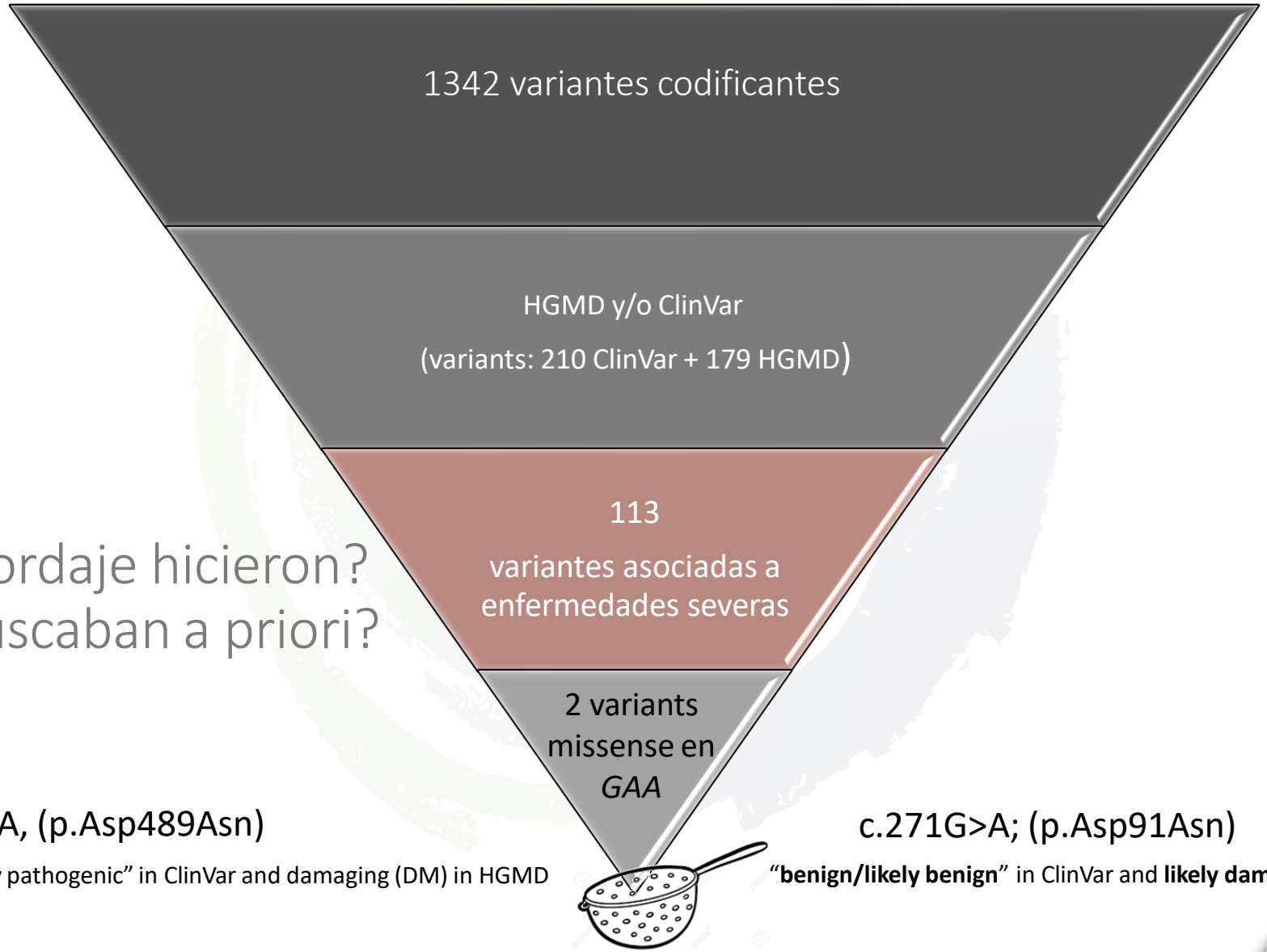
2015 (digitalis)

Dante Alighieri

We identified **two pathogenic variants** in the *GAA* gene, a genotype associated with late-onset POMPE disease

sanofi

NGS Variant filtering



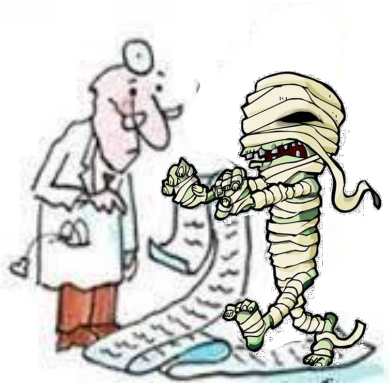
Que tipo de abordaje hicieron?
Sabían lo que buscaban a priori?

c.1465G>A, (p.Asp489Asn)

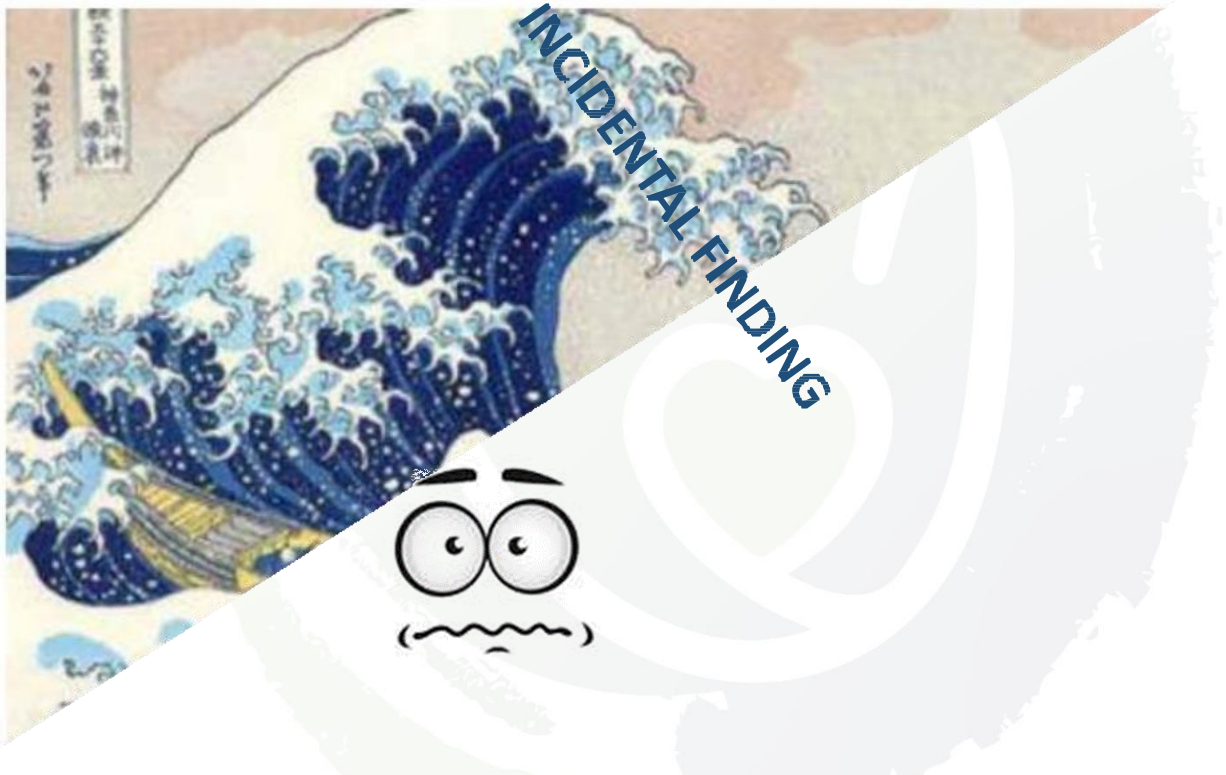
classified as "pathogenic/likely pathogenic" in ClinVar and damaging (DM) in HGMD

c.271G>A; (p.Asp91Asn)

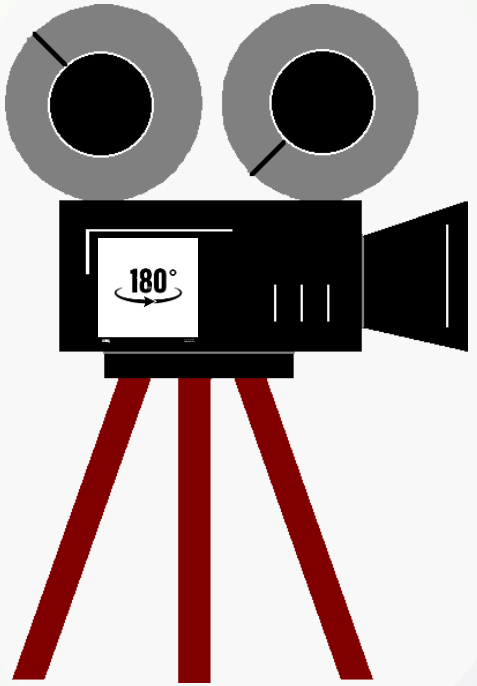
"**benign/likely benign**" in ClinVar and **likely damaging** in HGMD



NGS DATA TSUNAMI

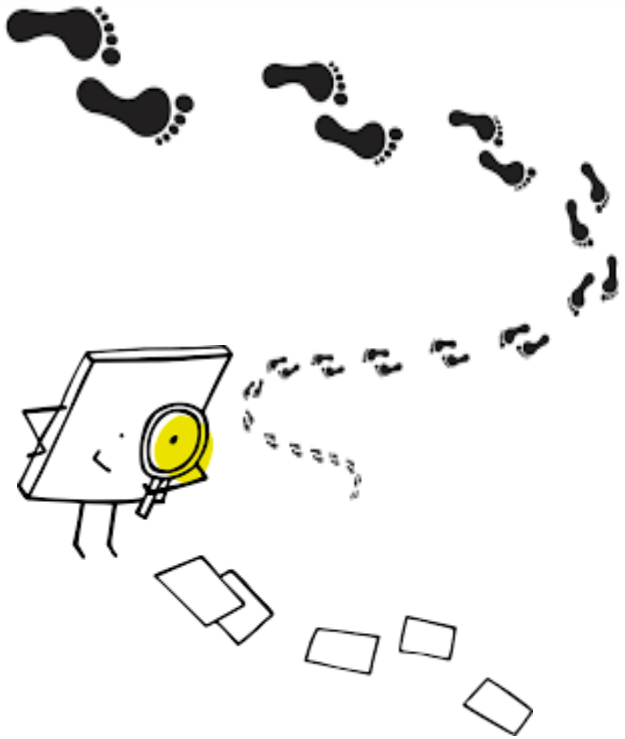


2015, (digitalis)

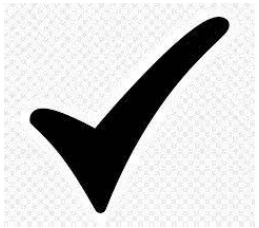


POMPE

2021



GAA(NM_001079804.3): c.1465G>A, (p.Asp489Asn)



ACMG Classification

Pathogenic

ClinVar **Pathogenic**

★★☆☆

Submissions: 7

Link to variant	Link to patients	Location	DNA nomenclature	RNA nomenclature	Protein nomenclature	Predicted severity	Phenotype with null allele	CRIM status
Variant info	Patients: 8	exon 10	c.1465G>A	r.(1465g>a)	p.(Asp489Asn)	Potentially less severe	Classic infantile	Positive

Variants

Link to Pubmed	PubMed
Location	exon 10
DNA nomenclature	c.1465G>A
RNA nomenclature	r.(1465g>a)
Protein nomenclature	p.(Asp489Asn)
Type of variant DNA	Substitution
Type of variant RNA	Substitution
Type of variant Protein	Substitution (missense)
ACMG classification	Likely pathogenic
Predicted severity	Potentially less severe
Phenotype with null allele	Classic infantile
CRIM status	Positive
MAF	MAF is less than 1%
RS number	rs398123169
Biochemical evidence of pathogenicity	decreased enzymatic activity in expression study
Splicing and translation prediction	no effect on splicing
Prediction of CRIM status	protein is expressed
Missense prediction (Mutation Taster)	Disease causing (p-value: 1)
Missense prediction (SIFT)	Deleterious (score: 0)

LOVD 3 — Global Variome shared LOVD

GAA (glucosidase, alpha; acid)

7 entries on 1 page. Showing entries 1 - 7.

100 per page — Legend — How to query

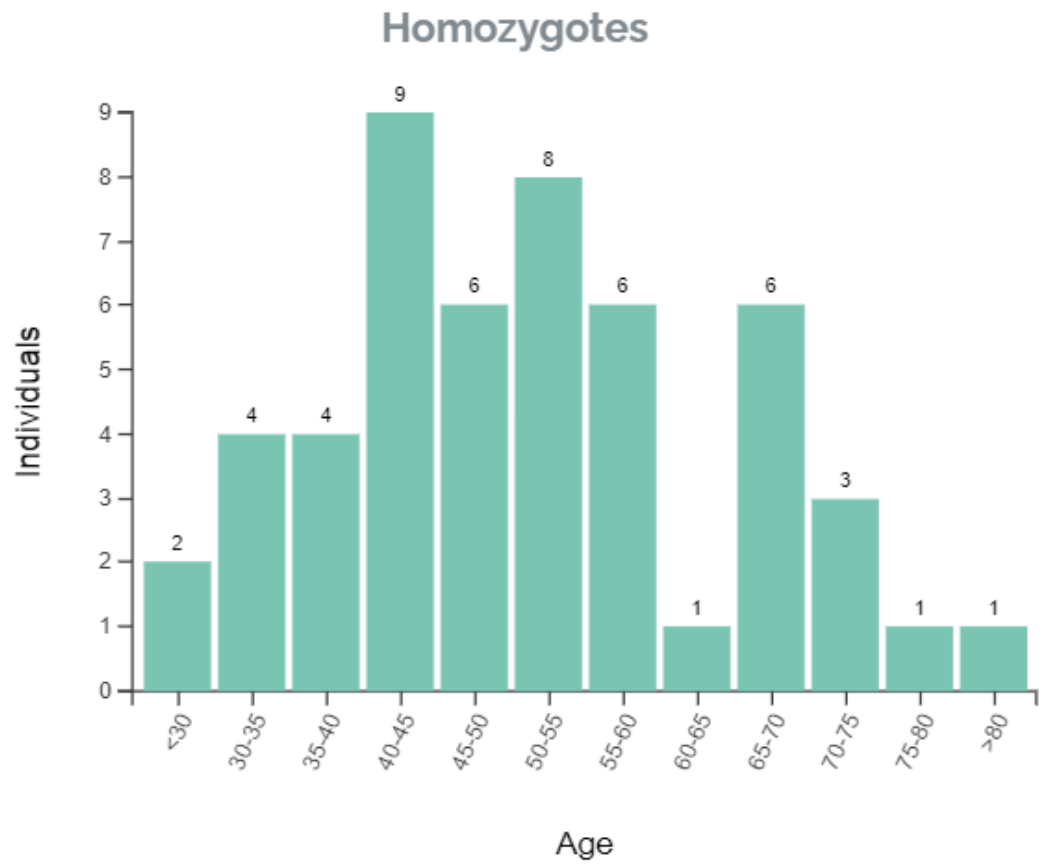
Effect	Exon	DNA change (cDNA)	Clinical classificatio
+/.	10	c.1465G>A	pathogenic (recessive)
+?/.	10	c.1465G>A	likely pathogenic
+?/.	10	c.1465G>A	likely pathogenic
+?/.	10	c.1465G>A	likely pathogenic
+?/.	10	c.1465G>A	likely pathogenic
+?/.	10	c.1465G>A	likely pathogenic
+?/.	10	c.1465G>A	likely pathogenic

sanofi

Frequencies

gnomAD Exomes Version: 2.1.1 $f = 0.0203$

Age Distribution



gnomAD Exomes

Allele Number	Homozygotes	Allele Frequency
15.980	-	0.00613
9.972	1	0.0138
18.326	-	0.000164
21.248	5	0.0178
111.706	63	0.0326
34.466	6	0.0118
30.548	4	0.00809
6.064	-	0.0218
248.310	**79**	**0.0203**
134.866	40	0.0201
113.444	39	0.0206

NM_000152.5(GAA):c.271G>A;p.(Asp91Asn)

www.pompecenter.nl
Erasmus MC
University Medical Center Rotterdam

(http://www. pompevariantdatabase.nl/)

Link to variant	Link to patients	Location	DNA nomenclature	RNA nomenclature	Protein nomenclature	Predicted severity	Phenotype with null allele	CRIM status
Variant info	Patients: 2	exon 2	c.271G>A	r.271g>a	p.(Asp91Asn)	Presumably non-pathogenic	Unknown (disease-associated)	Positive

LOVD 3 Leiden Open Variation Database
THE HUMAN VARIOME PROJECT
Global Variome shared LOVD
GAA (glucosidase, alpha; acid)
LOVD v.3.0 Build 27 [Current LOVD status]
Register as submitter | Log in

64 entries on 1 page. Showing entries 1 - 64.
100 per page Legend How to query

Effect	Exon	DNA change (cDNA)	RNA change	Protein	Enzyme activity	Allele	Classification method	Clinical classification	DNA
?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	VUS	g.780
?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	VUS	g.780
?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	VUS	g.780
?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	VUS	g.780
?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	VUS	g.780
-/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	benign	g.780
-?/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	likely benign	g.780
-?/.	-	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	likely benign	g.780
-/.	-	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	benign	g.780
-?/.	-	c.271G>A	r.(?)	p.(Asp91Asn)	-	Unknown	-	likely benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780
+/.	2	c.271G>A	r.(?)	p.(Asp91Asn)	-	Parent #1	-	benign	g.780

Más de 50 veces está como Benigna o probablemente benigna

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ESHG

ARTICLE

Enzymatic diagnosis of Pompe disease: lessons from 28 years of experience

Monica Y. Niño[1,2,3] · Mark Wijgerde[2] · Douglas Oliveira Soares de Faria[1,2,3] · Marianne Hoogeveen-Westerveld[2] · Atze J. Bergsma[1,2,3] · Mike Broeders[1,2,3] · Nadine A. M. E. van der Beek[3,4] · Hannerieke J. M. van den Hout[1,3] · Ans T. van der Ploeg[1,3] · Frans W. Verheijen[2] · W. W. M. Pim Pijnappel[1,2,3]

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Es una variante super conocida y reportada en varias publicaciones asociada a pseudodeficiencia.

In 794 individuals, two or more assays were performed. We found that phenotypes could only be distinguished using fibroblasts with 4MUG as substrate. Pseudodeficiencies caused by the *GAA2* allele could be ruled out using 4MUG rather than glycogen as substrate in leukocytes or fibroblasts. The Asian pseudodeficiency could only be ruled out in fibroblasts using 4MUG as substrate. We conclude that fibroblasts using 4MUG as substrate provides the most reliable assay for biochemical diagnosis and can serve to validate results from leukocytes or dried blood spots.

variants can lead to pseudodeficiency. In the Caucasian population, the *GAA2* (c.271G>A) pseudodeficiency variant lowers the activity of GAA for the natural substrate glycogen, but not for the artificial substrate 4MUG [27, 28].

Da baja la actividad Ez con glucogéno pero **con 4 MUG da normal**

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Pompe disease *GAA* variant database

List of variants | Advanced search | Reported patients | Export database | Help | c.271G>A

List of variants

Sort by Default | Displaying 1 - 1 of 1 | 100

Link to variant	Link to patients	Location	DNA nomenclature	RNA nomenclature	Protein nomenclature	Predicted severity	Phenotype with null allele	CRIM status
Variant info	Patients: 2	exon 2	c.271G>A	r.271g>a	p.(Asp91Asn)	Presumably non-pathogenic	Unknown (disease-associated)	Positive

Aún nadie describió paciente con las 2 variantes de la

Variants

Link to Pubmed	PubMed
Location	exon 2
DNA nomenclature	c.271G>A
RNA nomenclature	r.271g>a
Protein nomenclature	p.(Asp91Asn)
Type of variant DNA	Substitution
Type of variant RNA	Substitution
Type of variant Protein	Substitution (missense)
ACMG classification	Likely benign
Predicted severity	Presumably non-pathogenic
Phenotype with null allele	Unknown (disease-associated)
CRIM status	Positive
MAF	MAF is over 1%
RS number	rs1800299
Biochemical evidence of pathogenicity	lowers affinity for glycogen (GAA2)
Splicing and translation prediction	no effect on splicing
Prediction of CRIM status	protein is expressed
Missense prediction (Mutation Taster)	Polymorphism (p-value: 0)
Missense prediction (SIFT)	Tolerated (score: 0.08)

Patients

Link to patients	Allele 1 DNA	Allele 2 Location	Allele 2 DNA	Allele 2 Phenotype with a null allele	Phenotype of patient	Age of Onset	Gender	Age at analysis	Cardiomyopathy	Liver/ Spleen	Ventilatory support	Respiratory problems	Wheelchair dependency	Mobility problems	(Kypho) Scoliosis	Ptosis	Scapular winging	Cerebral vessels anomalies	No of patients reported	Country/P...
	c.271G>A		not disease-associated																0	
PubMed	c.271G>A	intron 1B	c.-32-13T>G	Childhood or Adult	Adult	47 years/28 years	F/M	50 years/36 years									-/+		2	Austria

Journal of Neurology (2018) 265:159–164
https://doi.org/10.1007/s00415-017-8686-6

ORIGINAL COMMUNICATION

CrossMark

Pompe disease in Austria: clinical, genetic and epidemiological aspects

W. N. Löscher[1] · M. Huemer[2] · T. M. Stulnig[3] · P. Simschitz[4] · S. Iglseder[5] · C. Eggers[5] · H. Moser[6] · D. Möslinger[7] · M. Freilinger[7] · F. Lagler[8] · S. Grinzinger[9] · M. Reichhardt[10] · R. E. Bittner[11] · W. M. Schmidt[11] · U. Lex[12] · M. Brunner-Krainz[13] · S. Quasthoff[14] · J. V. Wanschitz[1]

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Table 1 Demographic, clinical and genetic data of the Austrian LOPD patients

ID	Gender	Age	Symptom onset [years]	Findings at presentation	Diagnostic delay [years]	Duration of ERT [years]	Genetics allel 1	Genetics allel 2
1	M	36	21	l, a	6	8	c.-32-13T > G	c.877G > A
2	F	65	44	l, a, r	10	11	c.-32-13T > G	c.1912G > T
3	F	26	18	l, a, r	1	7	c.-32-13T > G	c.2281delinsAT
4	F	58	25	l, a, r	18	8	c.-32-13T > G	c.307T > G
4a	M	56	46	r	0	6	c.-32-13T > G	c.307T > G
6	F	37	22	l, a, r	1	8	c.-32-13T > G	**c.1076-2G > A**
7	M	27	11	l, a	5	11	c.692 + 5G > T	c.953T > C
8	F	29	18	l	0.5	11	c.-32-13T > G	c.877G > A
9	M	38	21	l, a, r, s	0.5	11	c.-32-13T > G	c.1051delG
10	F	64	47	l	3	12	c.-32-13T > G	c.271G > A
11	M	62	24	l, a, s	34	3	c.-32-13T > G	c.1051delG
13	M	39	nk	l, a, r	nk	*	c.-32-13T > G	c.955 + 2T > G
14	M	37	28	l, a, s	8	1	c.-32-13T > G	c.271 G > A
15	M	16	3.5	l, a	1.5	11	c.1076-22T > G	c.525delT
16	M	13	6	l, a, s	6.5	1	c.1548G > A	**c.1470C > A**
17	F	69	40	l, a, r	28	**	c.-32-13T > G	**c.323G > C**
18	F	25	nk	l, a	nk	**	c.-32-13T > G	c.2608C > T
19	M	15	15	HyperCK	0.5	0	c.-32-13T > G	**c.2380dupC**
20	M	11	8	HyperCK	3	0	c.-32-13T > G	c.2051C > G
21	W	13	12.5	HyperCK	0.5	0	c.1134C > G	c.1478C > T

New mutations are bold

nk not known, *l* limb-girdle weakness, *a* axial weakness, *r* respiratory weakness, *s* scapula alata, *HyperCK* asymptomatic hyperCKemia, *ERT* enzyme replacement therapy

* pt. decided to stop treatment after 2 years due to lack of efficacy and severe disease, ** ERT recently started

Estarán en TRANS???

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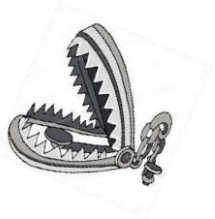
Case Reports > Front Pediatr. 2022 Jun 1;10:855510. doi: 10.3389/fped.2022.855510.
eCollection 2022.

In addition, the missense variant c.271G>A listed as “presumably non-pathogenic” in the Pompe center database, a conclusion based on previous *in vitro* expression experiments of GAA protein, showed significant *GAA* exon 2 skipping with 30% reduction of exon 2 inclusion (Fig. 2E-F lane 8). However, this variant is a very well known *GAA* polymorphism and no evidence of reduced GAA enzymatic activity has been reported in subjects presenting the c.271G>A variant in the homozygous condition. This may not be totally unexpected, since it is likely that a 30% reduction of correctly spliced *GAA* transcript may go unnoticed within the broad range of GAA activity found in normal subjects. Nonetheless, although the c.271G>A variant may not be pathogenic a possible role in *GAA* exon 2 skipping has to be taken into account and a splicing pattern analysis in subjects presenting the c.271G>A variant might be further investigated.

OJO, se sabe que afecta el splicing del exón 2.
Falta conocer más aun de esta variante.

Como verán a esta altura hay muchas dudas

Biochemical evidence of pathogenicity	lowers affinity for glycogen (GAA2)
Splicing and translation prediction	no effect on splicing
Prediction of CRIM status	protein is expressed
Missense prediction (Mutation Taster)	Polymorphism (p-value: 0)
Missense prediction (SIFT)	Tolerated (score: 0.08)



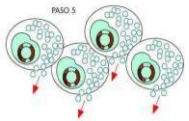
Confirmació
Genétic

c.1465G>A,
(p.Asp489Asn):
PROBABLEMENTE
PATOGÉNICA

DIAGNÓSTICO DE POMPE ????

Confirmación
enzimática

c.271G>A
(p.Asp91Asn):
pseudodeficiencia
Benigna/Prob benig



Confirmación
Genética + Enzimática

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fuente?

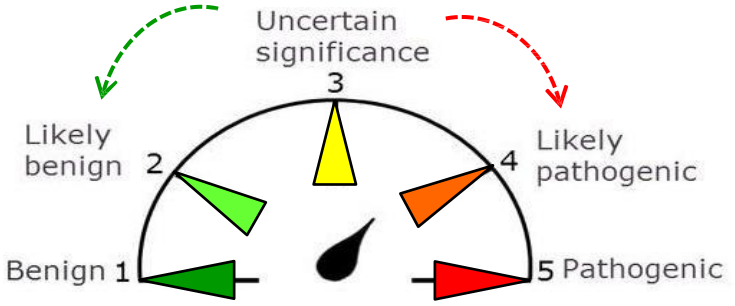
Accionar médico?

Que no encuentre la 2da variante significa que el paciente no tiene POMPE

Muchas de las alteraciones no detectadas están en zonas que no se analizan x NGS ni MLPA

- Mutaciones intrónicas profundas que afectan splicing
- Inserciones en intrones (transposón) (Bychkov et al, Int J Mol Sci. 2021)
- Variantes silenciosas que se descartan, pero afectan splicing (ej, última base del exón 2)
- Inversiones
- Translocaciones
- Variantes en promotor y sitios UTR que sin ensayos funcionales no pueden corroborarse
- Alteraciones epigenéticas como la metilación del ADN, las modificaciones de histonas y los microARN

QUÉ PUEDO HACER?



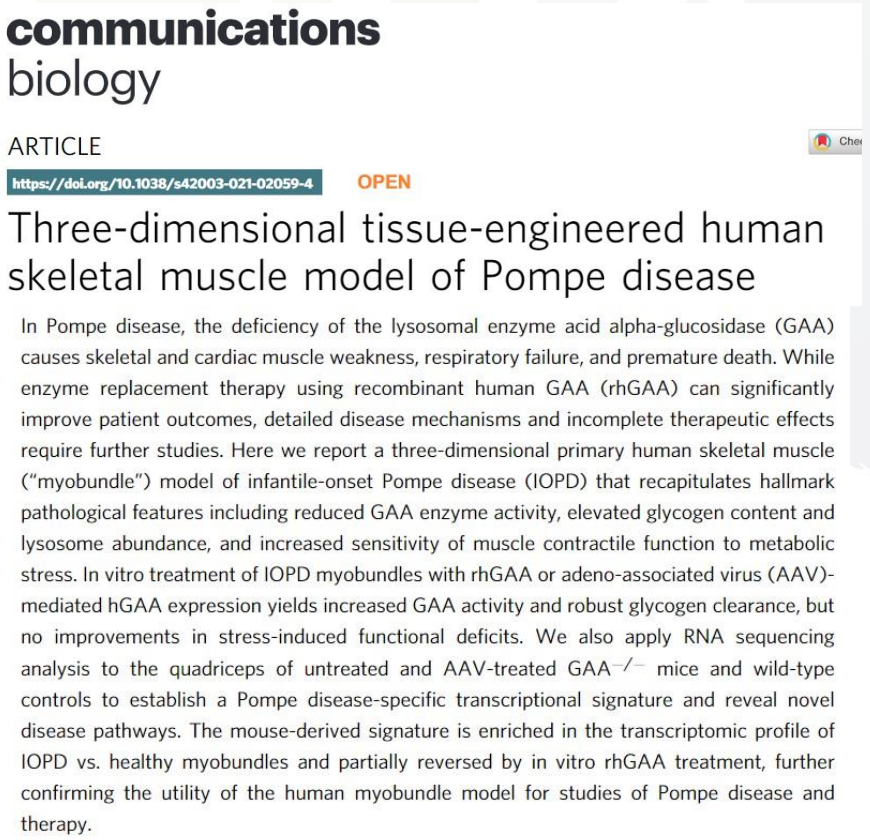
ARNm, qPCR, WGS, Biopsia

- Puedo obtener ARNm de sangre (Leucocitos): alteraciones de splicing intrónicas profundas
- qPCR para cuantificar expresión y evaluar cantidad de ARNm: no hay alteración de splicing pero tengo poco o nulo ARNm) ej:Caso de transposón (TE) o alteraciones epigenéticas (metilación, microRNA).
- WGS (detecto alteración intrónica profunda, ej TE)
- Biopsia de músculo (útil en ausencia de una segunda variante para respaldar el diagnóstico de la enfermedad al mostrar la acumulación patógena de glucógeno)

Transcriptoma

(para investigación)

Transcriptomic analysis of GAA−/−(KO) mice and identifi-cation of a Pompe disease signature:
Transcriptomic analysis revealed 2244genes that were significantly differentially expressed



communications biology

ARTICLE

https://doi.org/10.1038/s42003-021-02059-4 OPEN

Three-dimensional tissue-engineered human skeletal muscle model of Pompe disease

In Pompe disease, the deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA) causes skeletal and cardiac muscle weakness, respiratory failure, and premature death. While enzyme replacement therapy using recombinant human GAA (rhGAA) can significantly improve patient outcomes, detailed disease mechanisms and incomplete therapeutic effects require further studies. Here we report a three-dimensional primary human skeletal muscle ("myobundle") model of infantile-onset Pompe disease (IOPD) that recapitulates hallmark pathological features including reduced GAA enzyme activity, elevated glycogen content and lysosome abundance, and increased sensitivity of muscle contractile function to metabolic stress. In vitro treatment of IOPD myobundles with rhGAA or adeno-associated virus (AAV)-mediated hGAA expression yields increased GAA activity and robust glycogen clearance, but no improvements in stress-induced functional deficits. We also apply RNA sequencing analysis to the quadriceps of untreated and AAV-treated GAA$^{-/-}$ mice and wild-type controls to establish a Pompe disease-specific transcriptional signature and reveal novel disease pathways. The mouse-derived signature is enriched in the transcriptomic profile of IOPD vs. healthy myobundles and partially reversed by in vitro rhGAA treatment, further confirming the utility of the human myobundle model for studies of Pompe disease and therapy.

TRABAJAR INTERDISCIPLINARIAMENTE

-Recordar que puede ser muy difícil en ciertos casos encontrar las variantes

-No descartar POMPE si el estudio genético no arroja resultados concluyentes

-Confiar en la clínica: Usar las otras herramientas para arribar al Diagnóstico

CONSULTAR

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DISEASES
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¡Muchas gracias por su atención!

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MAT-CO-2202507 08/2022