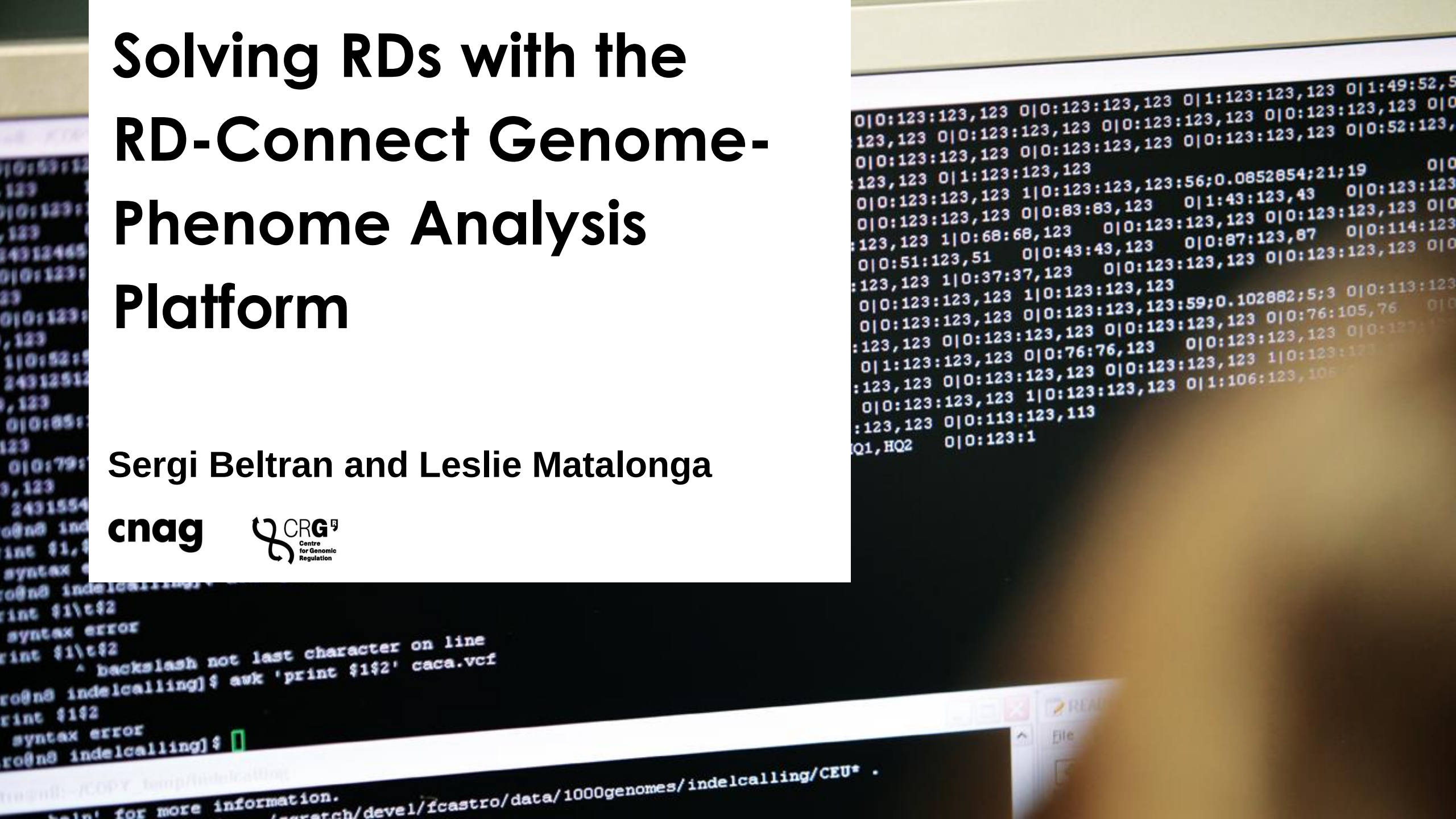
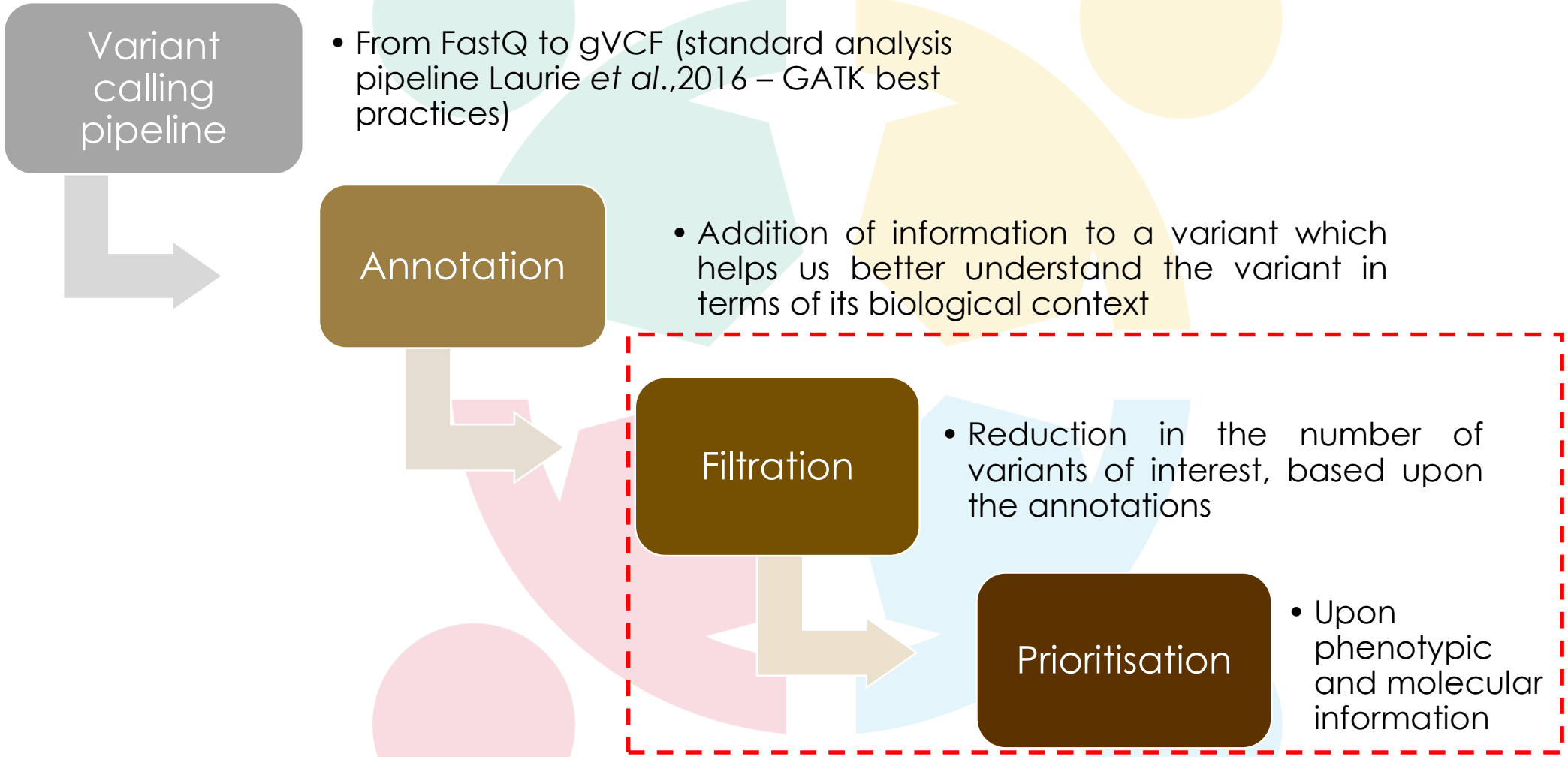
Solving RDs with the RD-Connect Genome-Phenome Analysis Platform

Sergi Beltran and Leslie Matalonga

cnag CRG Centre for Genomic Regulation

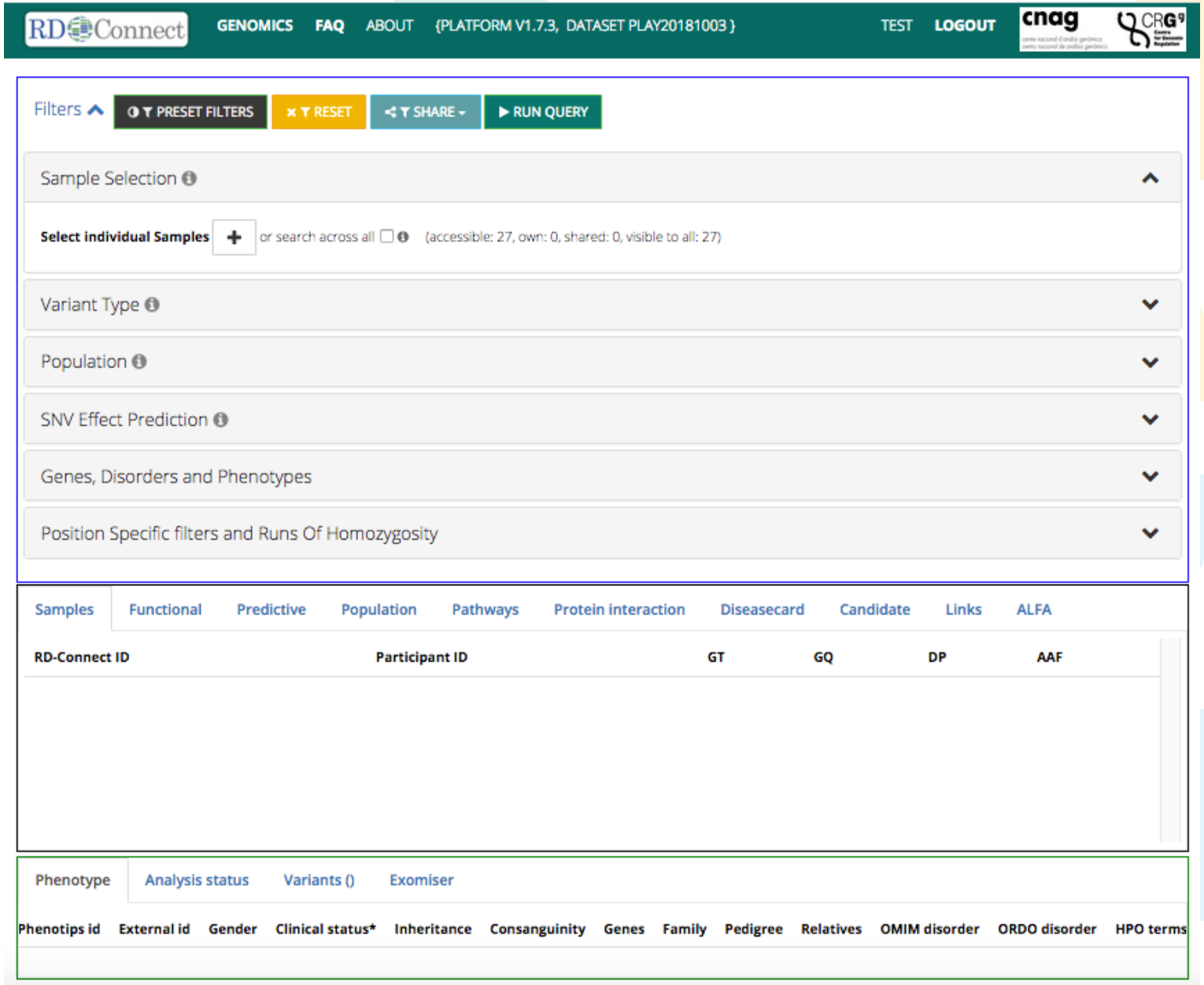


Molecular diagnostic and gene discovery challenge



Funded by the European Union
GA n°825575

Genome - Phenome Analysis Platform



1. SAMPLES

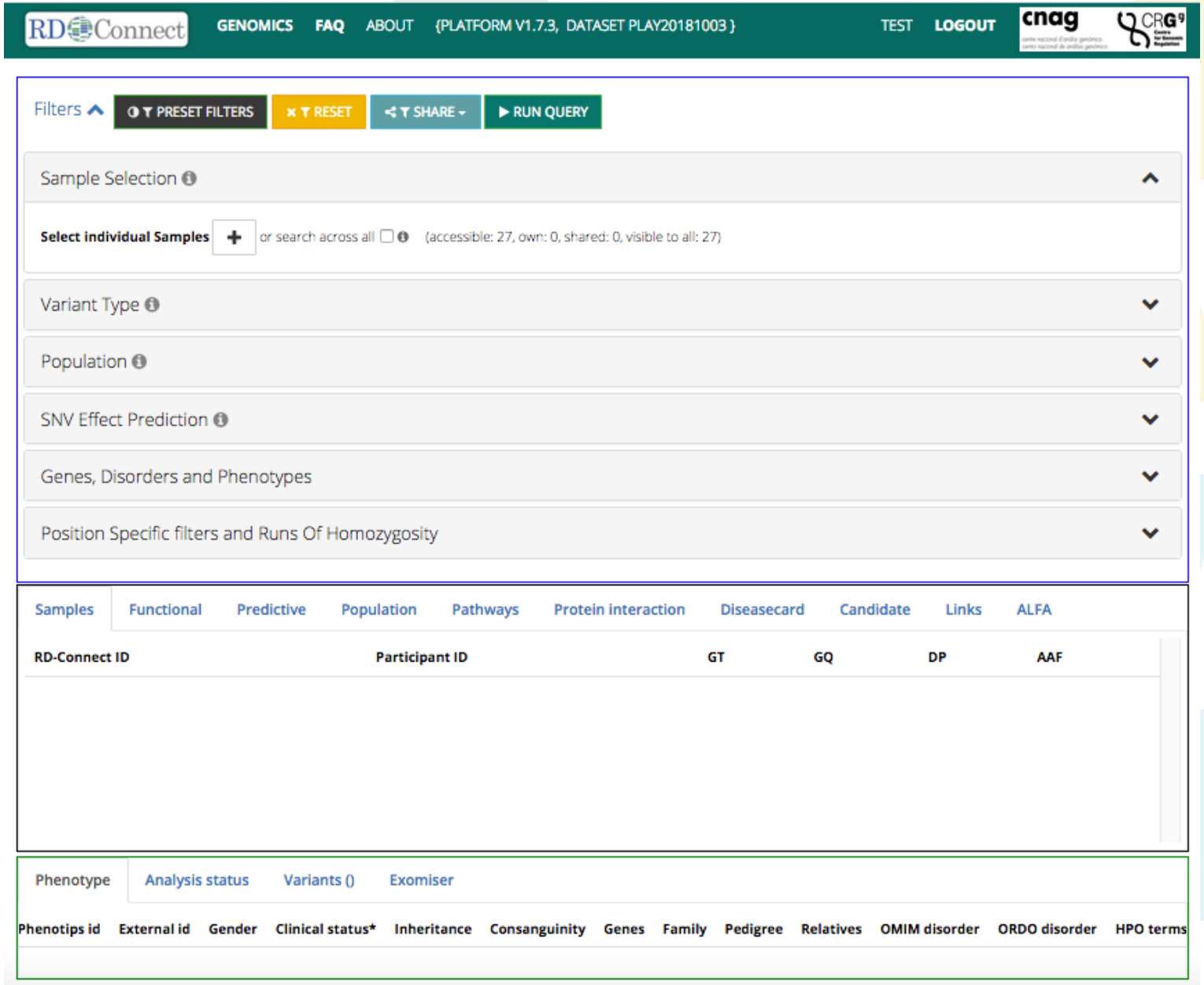
2. FILTERS

3. RESULTS

EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico
CRG Centre for Genomic Regulation
Funded by the European Union GA n°825575

Genome - Phenome Analysis Platform



1. SAMPLES

2. FILTERS

3. RESULTS

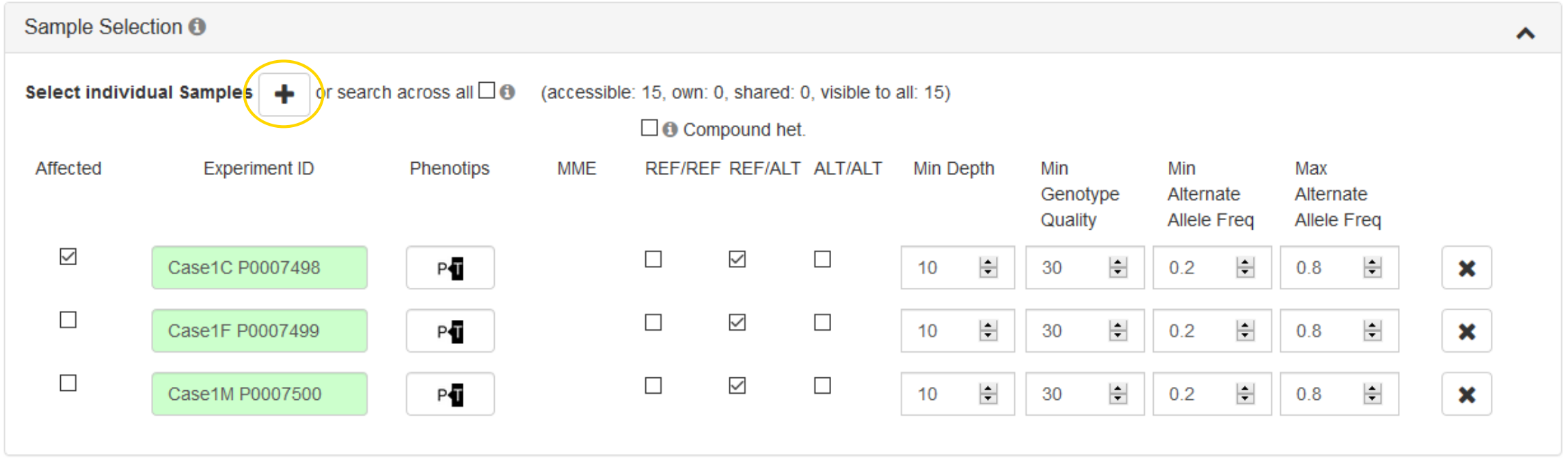
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Samples, inheritance and quality parameters



Genotypes – mode of inheritance

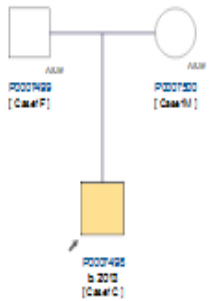
Quality parameters

Funded by the European Union GA n°825575

Samples, inheritance and quality parameters

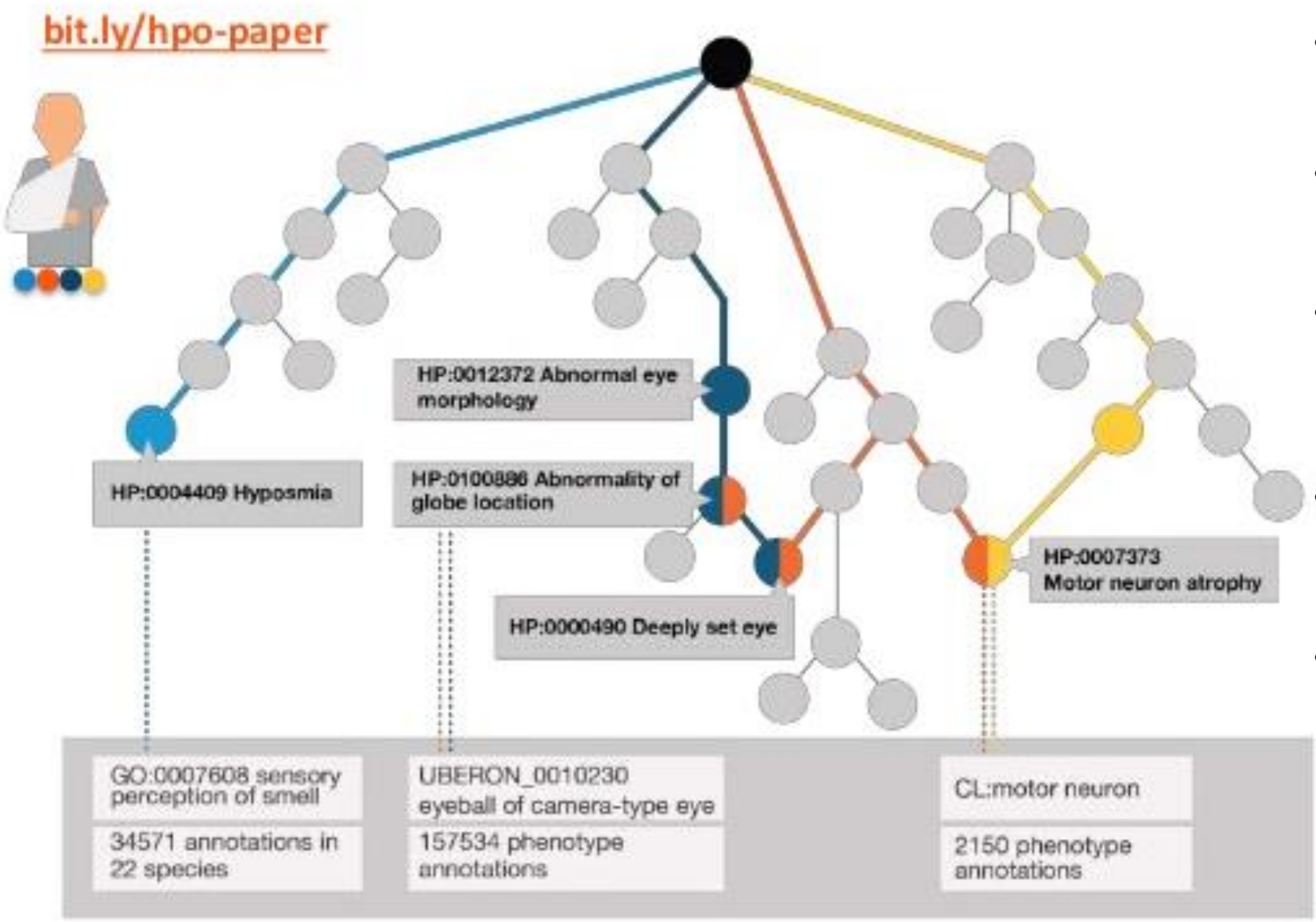
Summary of the patient phenotypic information

Phenotype | Analysis status | Variants () | Exomiser

Phenotips id	External id	Gender	Clinical status*	Inheritance	Consanguinity	Genes	Family	Pedigree	ORDO disorder	HPO terms
P0007498	Case1C	Male	Affected	Sporadic	false	unknown	FAM0001814		Congenital myopathy	Neck muscle weakness Muscular hypotonia Neonatal hypotonia Congenital hip dislocation Inability to walk Recurrent lower respiratory tract infections Arthrogryposis multiplex congenita Skeletal muscle atrophy Distal arthrogryposis Weakness of facial musculature NO Intellectual disability

Funded by the European Union GA n°825575

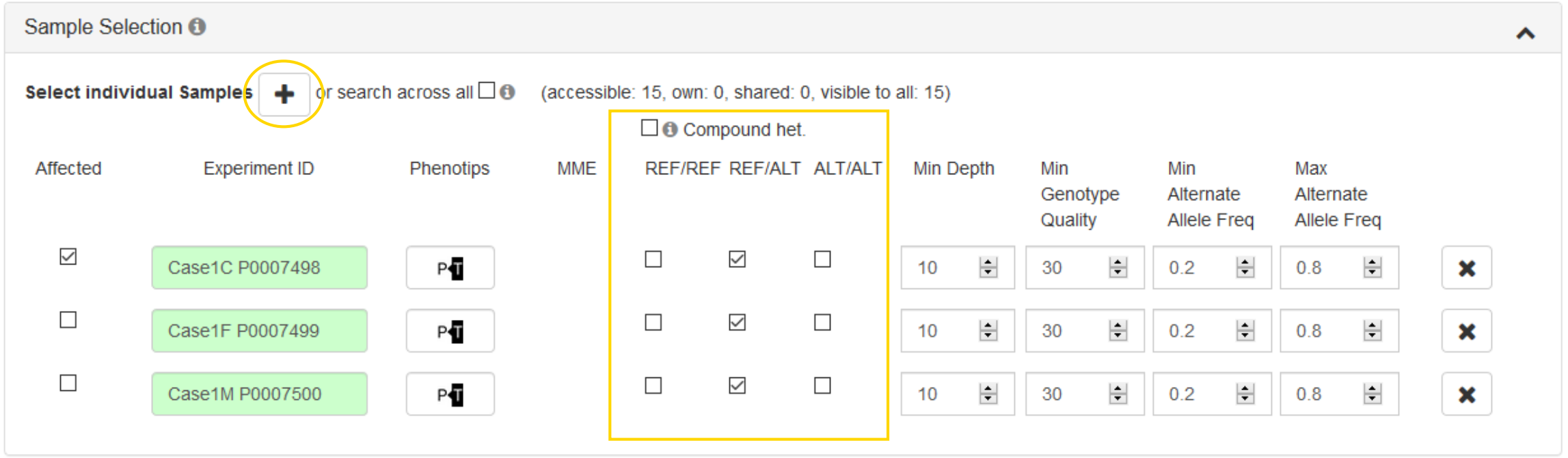
Human Phenotype ontology: what is an HPO?



- Standardised vocabulary of phenotypic abnormalities
- Tree based classification – symptom granularity
- Developed using medical literature: Orphanet, OMIM and Decipher
- Terms are mapped with other ontologies (e.g. GO)
- Currently comprises more than 13,000 terms and over 156,000 annotations to hereditary diseases

EJP RD
cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico
CRG Centre for Genomic Regulation
Funded by the European Union GA n°825575

Samples, inheritance and quality parameters

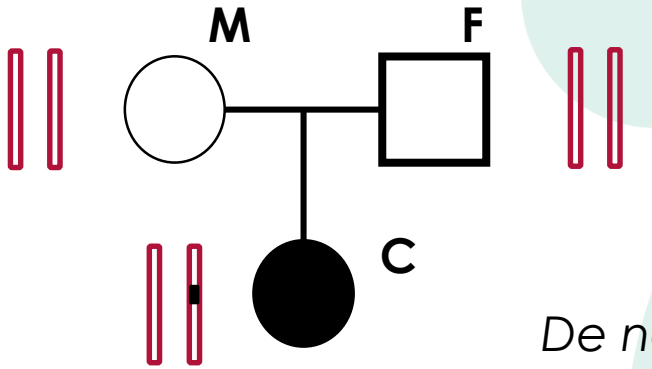


Genotypes – mode of inheritance

Quality parameters

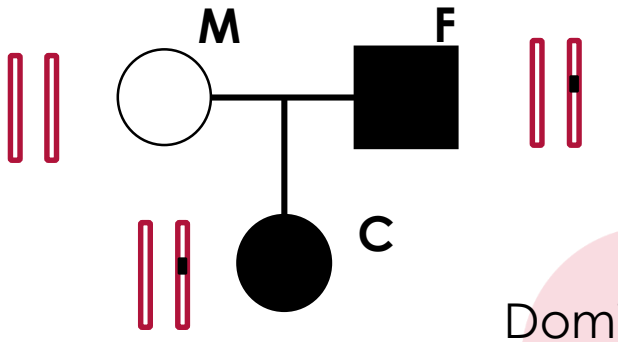
Funded by the European Union GA n°825575

Genotypes and mode of inheritance



De novo

Affected	Experiment ID	REF/REF	REF/ALT	ALT/ALT
☑	Index case	☐	☑	☐
☐	Mother	☑	☐	☐
☐	Father	☑	☐	☐



Dominant

Affected	Experiment ID	REF/REF	REF/ALT	ALT/ALT
☑	Index case	☐	☑	☐
☐	Mother	☑	☐	☐
☑	Father	☐	☑	☐

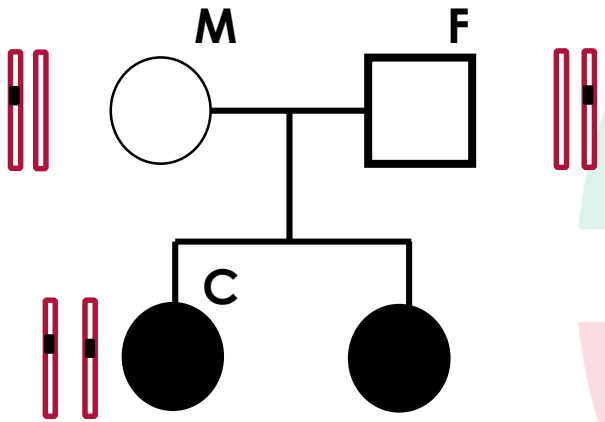
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Genotypes and mode of inheritance



Autosomal recessive

Affected	Experiment ID	REF/REF	REF/ALT	ALT/ALT
☑	Index case	☐	☐	☑
☐	Mother	☐	☑	☐
☐	Father	☐	☑	☐

☑ Compound het.

Affected	Experiment ID	REF/REF	REF/ALT	ALT/ALT
☑	Index case	☐	☑	☐
☐	Mother	☑	☑	☐
☐	Father	☑	☑	☐

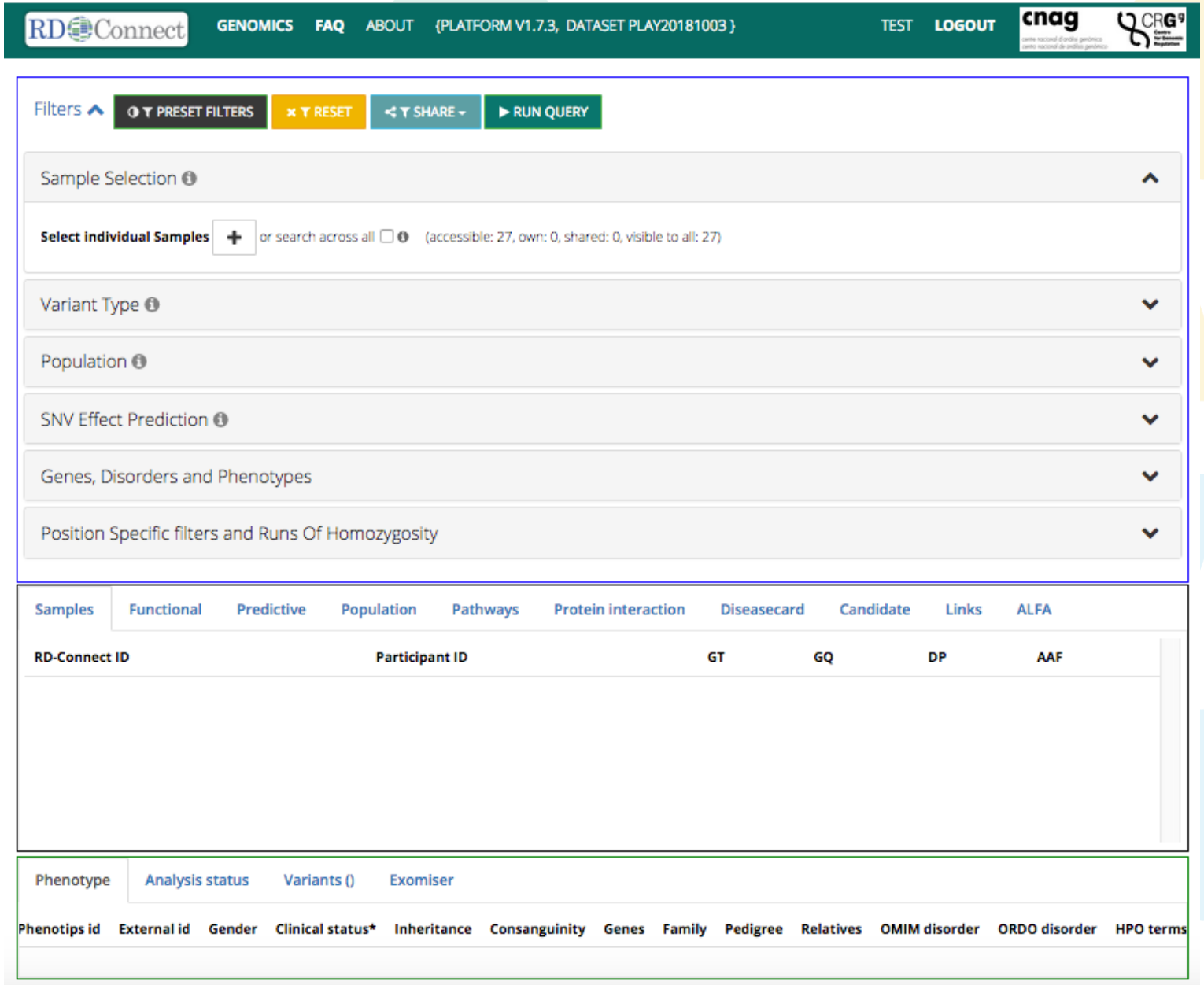
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Genome - Phenome Analysis Platform



1. SAMPLES

2. FILTERS

3. RESULTS

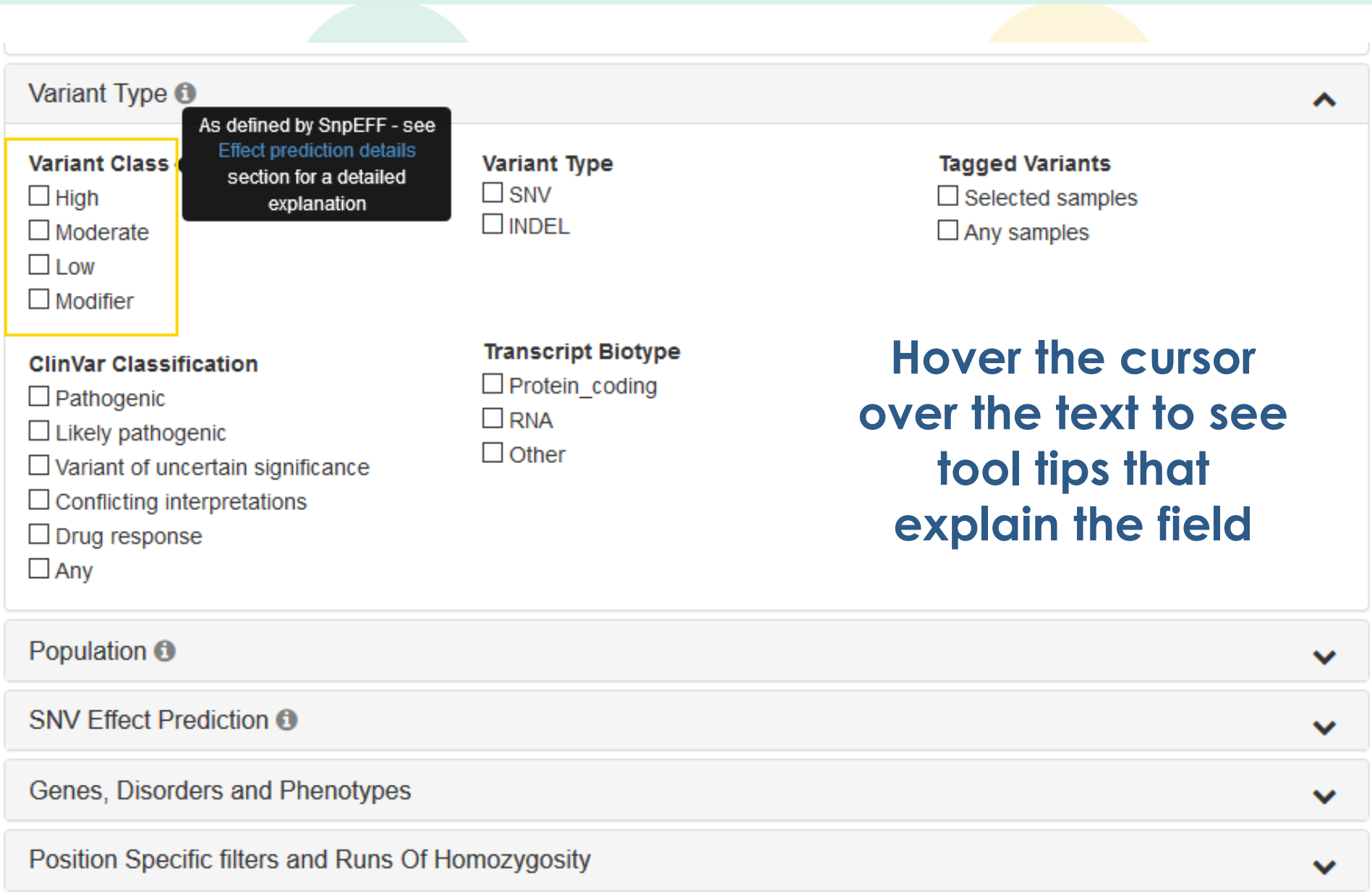
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Filtering steps

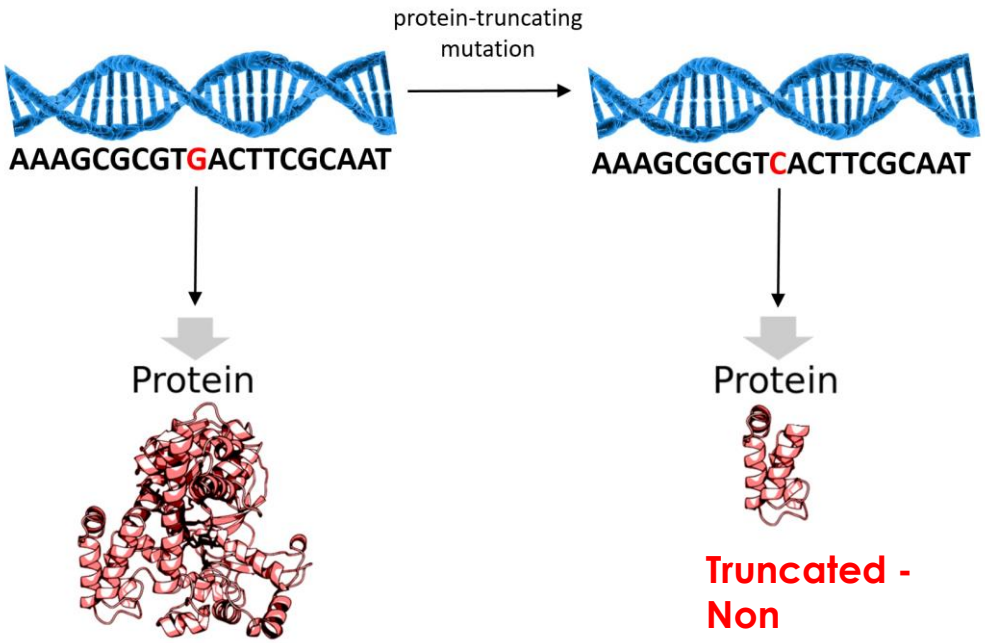


EJP RD
cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico
CRG Centre for Genomic Regulation
Funded by the European Union GA n°825575

Filtering steps- variant consequence

SnpEff/ VEP categorise all variants into one of four impact categories (variant consequence annotation)

HIGH	Variants significantly **affect protein structure** e.g. nonsense, frameshift, canonical splice-sites
MODERATE	Mainly **non-synonymous** variants i.e. amino-acid change
LOW	Mainly **synonymous** variants i.e. no amino acid change
MODIFIER	Mutations in **non-coding** regions



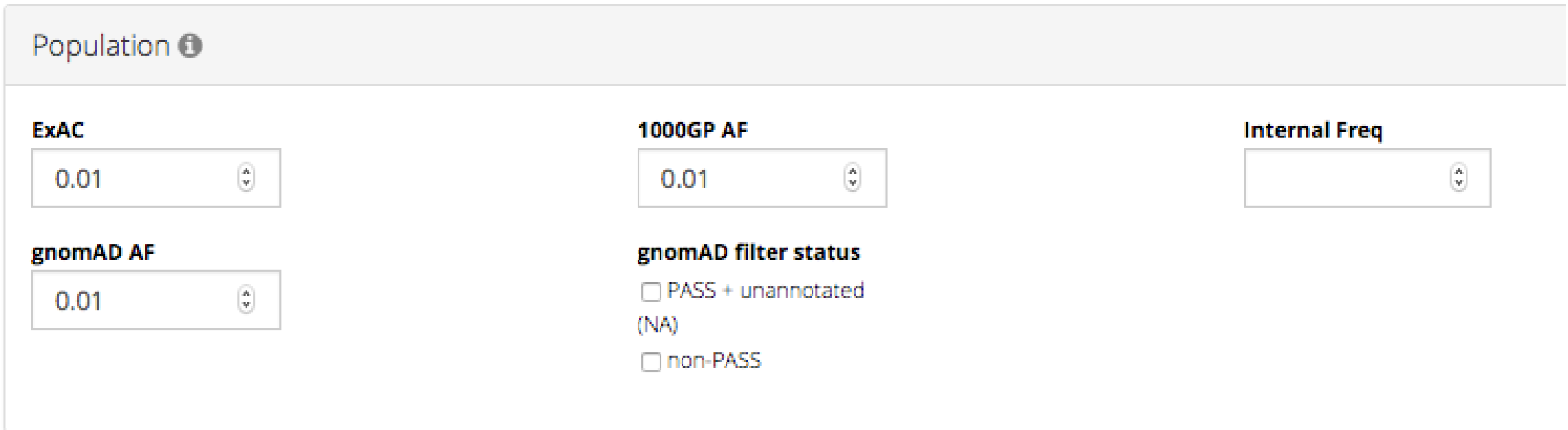
Source: http://thedishonscience.stanford.edu/posts/essential-genes/

Funded by the European Union GA n°825575

Filtering steps, tools and databases

Databases of allele frequencies in ***control*** populations allows us to estimate how common or rare a particular variant may be.

FILTER FOR VARIANT PRESENT IN LESS THAN 1% OF ALLELES



Funded by the European Union GA n°825575

Filtering steps, tools and databases

Filter using a list of candidate genes generated on-the-fly

Genes in the panel: ACTA1, BIN1, CACNA1S, CFL2, COL12A1, COL6A1, COL6A2, COL6A3, DNM2, EPG5, FKBP14, HTRA2, KBTBD13, KLHL40, KLHL41, LGI4, LMNA, LMOD3, MAP3K20, MEGF10, MICU1, MTM1, MYH2, MYH3, MYH7, MYH8, MYMK, MYO18B, NEB, ORAI1, RYR1, SCN4A,

OWN CANDIDATE GENES

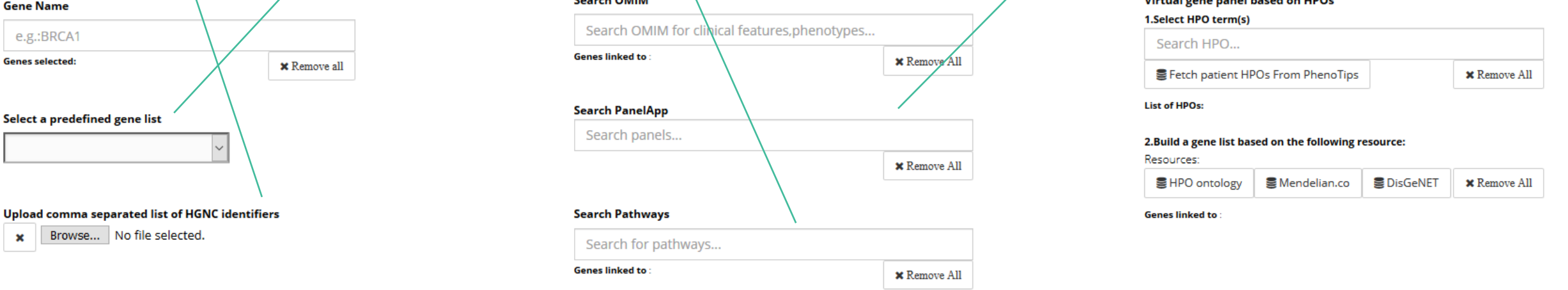
PUBLISHED GENE LISTS

PATHWAYS

SPECIFIC DISEASE

GROUP OF DISORDERS

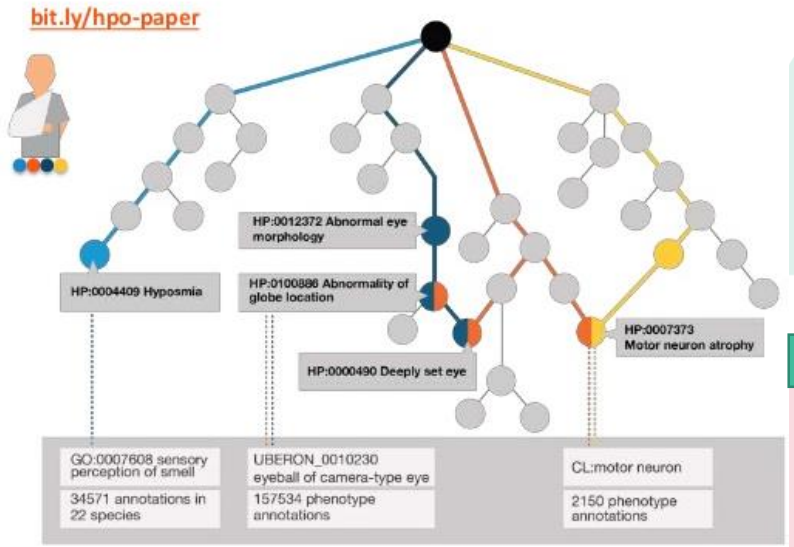
PATIENT SYMPTOMS



Funded by the European Union GA n°825575

Filtering steps, tools and databases

Filter using a list of candidate genes generated on-the-fly



PATIENT SYMPTOMS

Virtual gene panel based on HPOs

1.Select HPO term(s)

Search HPO...

Fetch patient HPOs From PhenoTips | Remove All

List of HPOs:

2.Build a gene list based on the following resource:

Resources:

HPO ontology | Mendelian.co | DisGeNET | Remove All

Genes ... to :

EJP RD

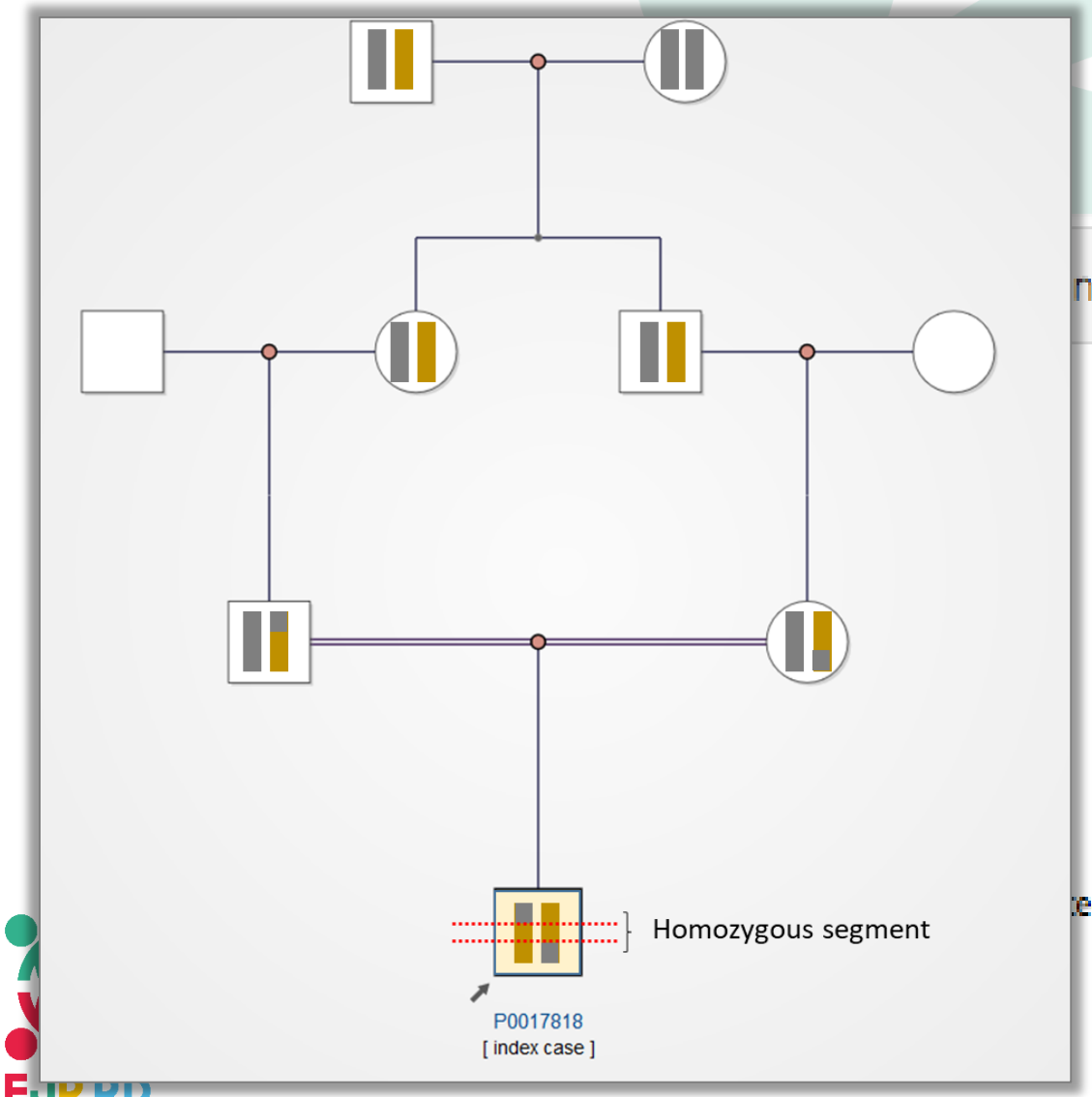
cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

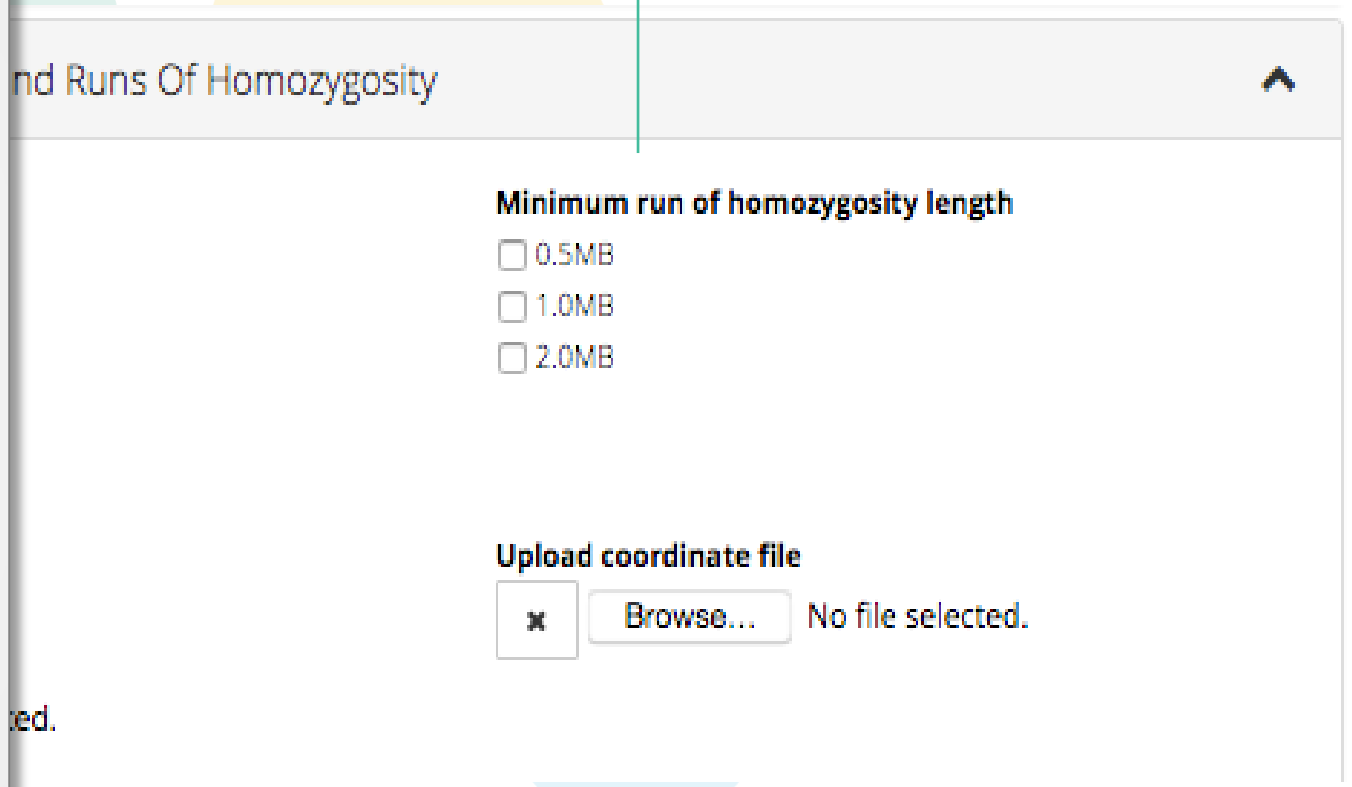
Funded by the European Union GA n°825575

Filtering steps, tools and databases

Filter to Regions of Interest (ROI) and/or regions containing long Runs of Homozygosity (Absence of Heterozygosity)



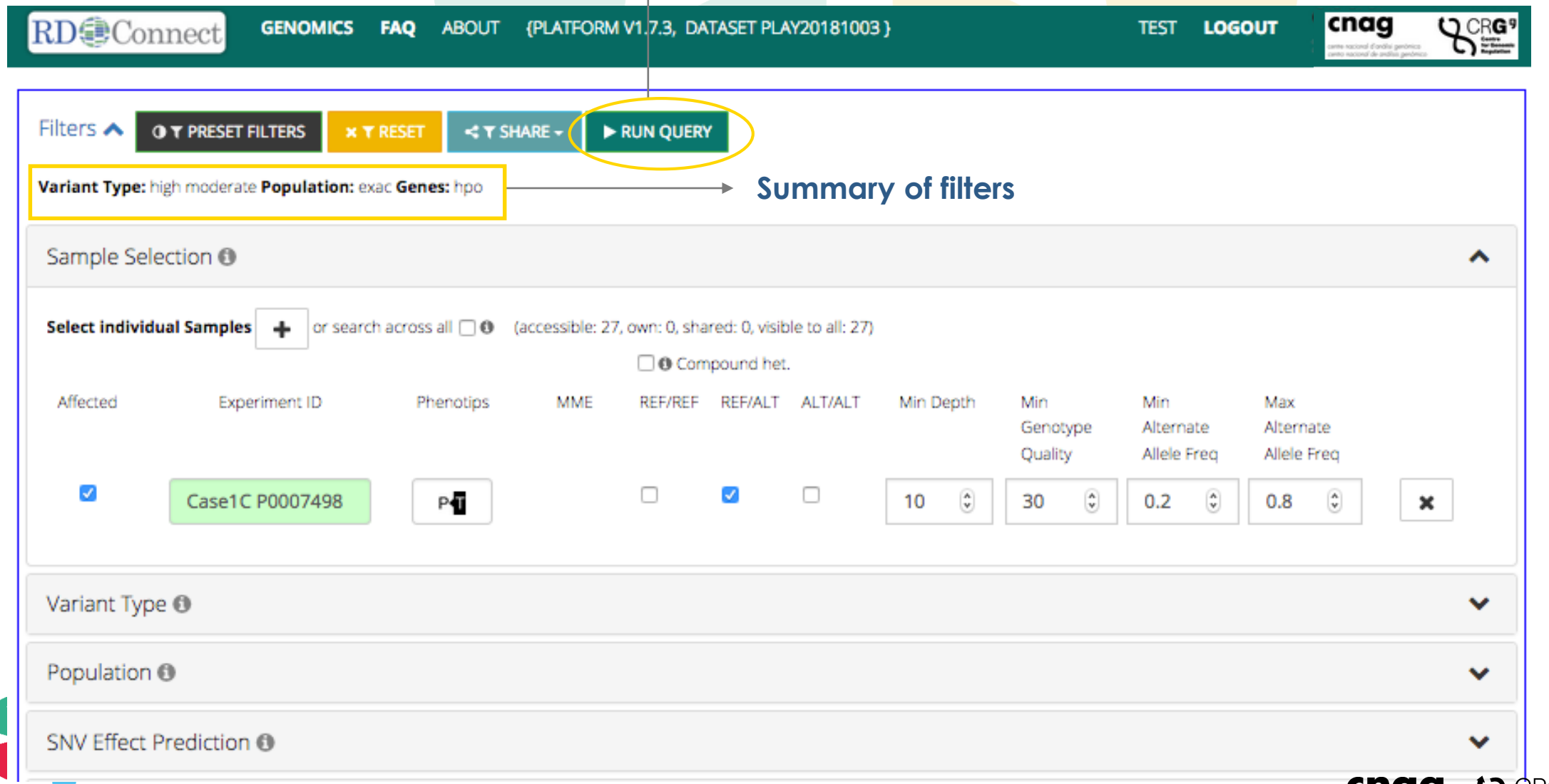
Filter to only variants found within a RoH- particularly useful when consanguinity is suspected.



Funded by the European Union GA n°825575

Filtering steps, tools and databases

RUN your QUERY to see the results



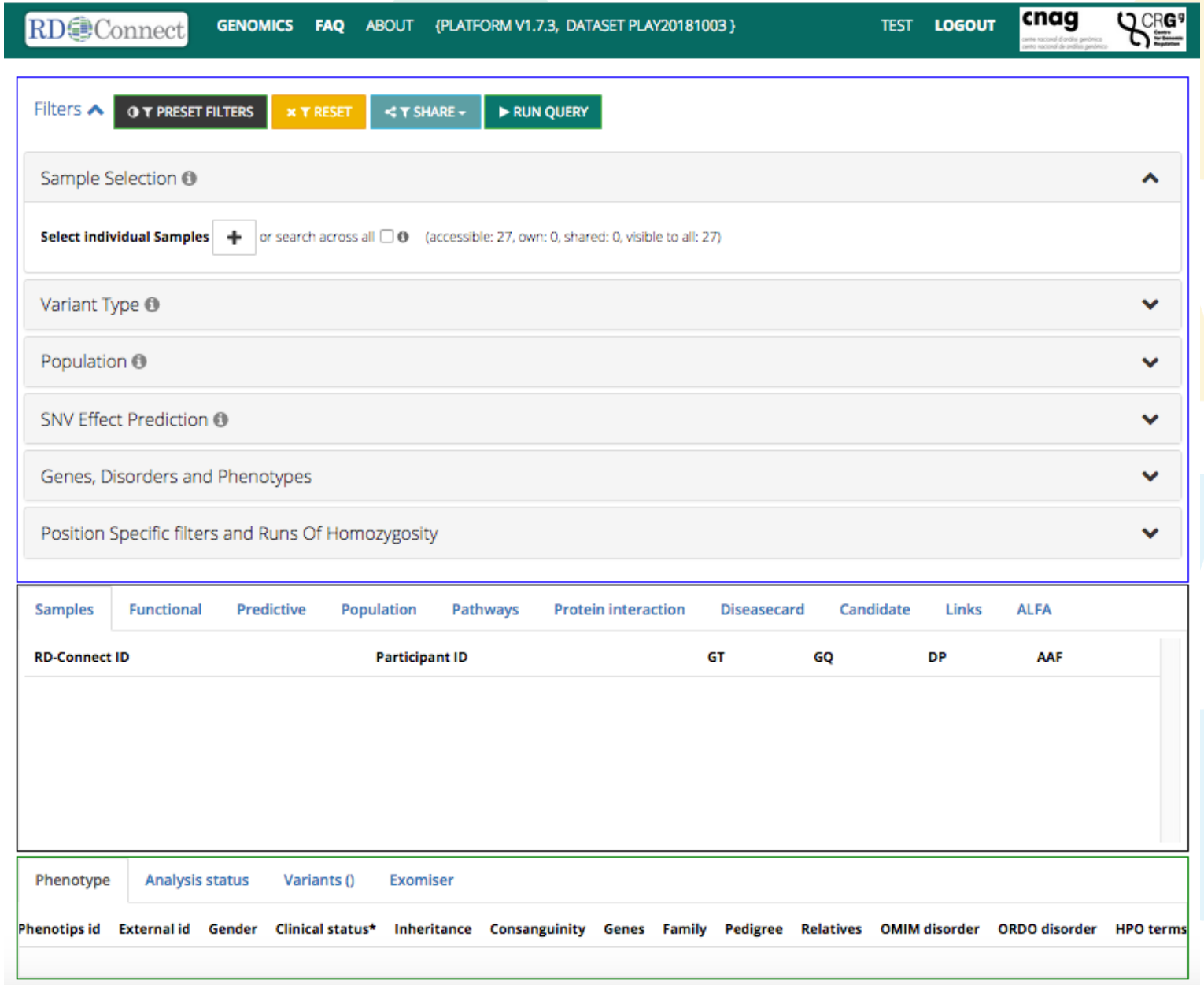
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Genome - Phenome Analysis Platform



1. SAMPLES

2. FILTERS

3. RESULTS

EJP RD

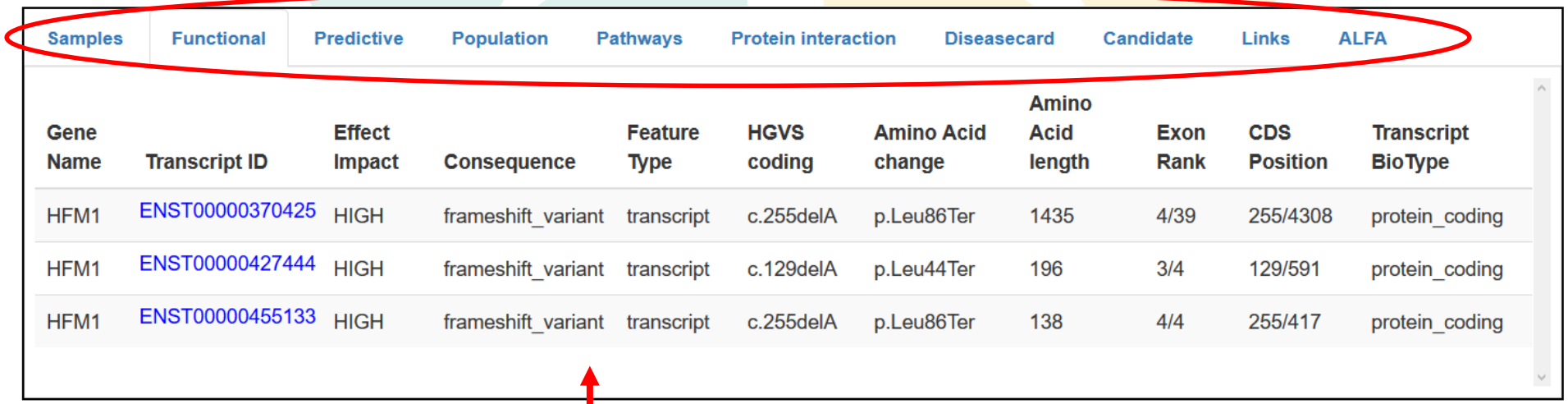
cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Results section

Multiple tabs with detailed information for data interpretation



Samples | Functional | Predictive | Population | Pathways | Protein interaction | Diseasecard | Candidate | Links | ALFA

Gene Name	Transcript ID	Effect Impact	Consequence	Feature Type	HGVS coding	Amino Acid change	Amino Acid length	Exon Rank	CDS Position	Transcript BioType
HFM1	ENST00000370425	HIGH	frameshift_variant	transcript	c.255delA	p.Leu86Ter	1435	4/39	255/4308	protein_coding
HFM1	ENST00000427444	HIGH	frameshift_variant	transcript	c.129delA	p.Leu44Ter	196	3/4	129/591	protein_coding
HFM1	ENST00000455133	HIGH	frameshift_variant	transcript	c.255delA	p.Leu86Ter	138	4/4	255/417	protein_coding

Phenotype | Analysis status | Variants (22) | Exomiser

First | Previous | 1 | Next | Last

Chr	Position	dbSNP	Ref	Alt	Candidate	GT Case1C	GT Case1F	GT Case1M	INDEL	Gene	Effect Impact	ClinVar	CADD	SIFT	PP2	MT	ExAC	1000GP AF	gnomAD AF	Internal Freq
1	91859888	.	GT	G	0 TAG	GT/G	GT/GT	GT/GT	☑	HFM1	HIGH		< 20				NA	NA	NA	0.166667
1	225477618	.	G	C	0 TAG	G/C	G/G	G/G		DNAH14	MODERATE		23.1	D	P	N	NA	NA	NA	0.166667
2	233349186	.	G	A	0 TAG	G/A	G/G	G/G		ECEL1	MODERATE		23.4	D	B	N	NA	NA	NA	0.166667

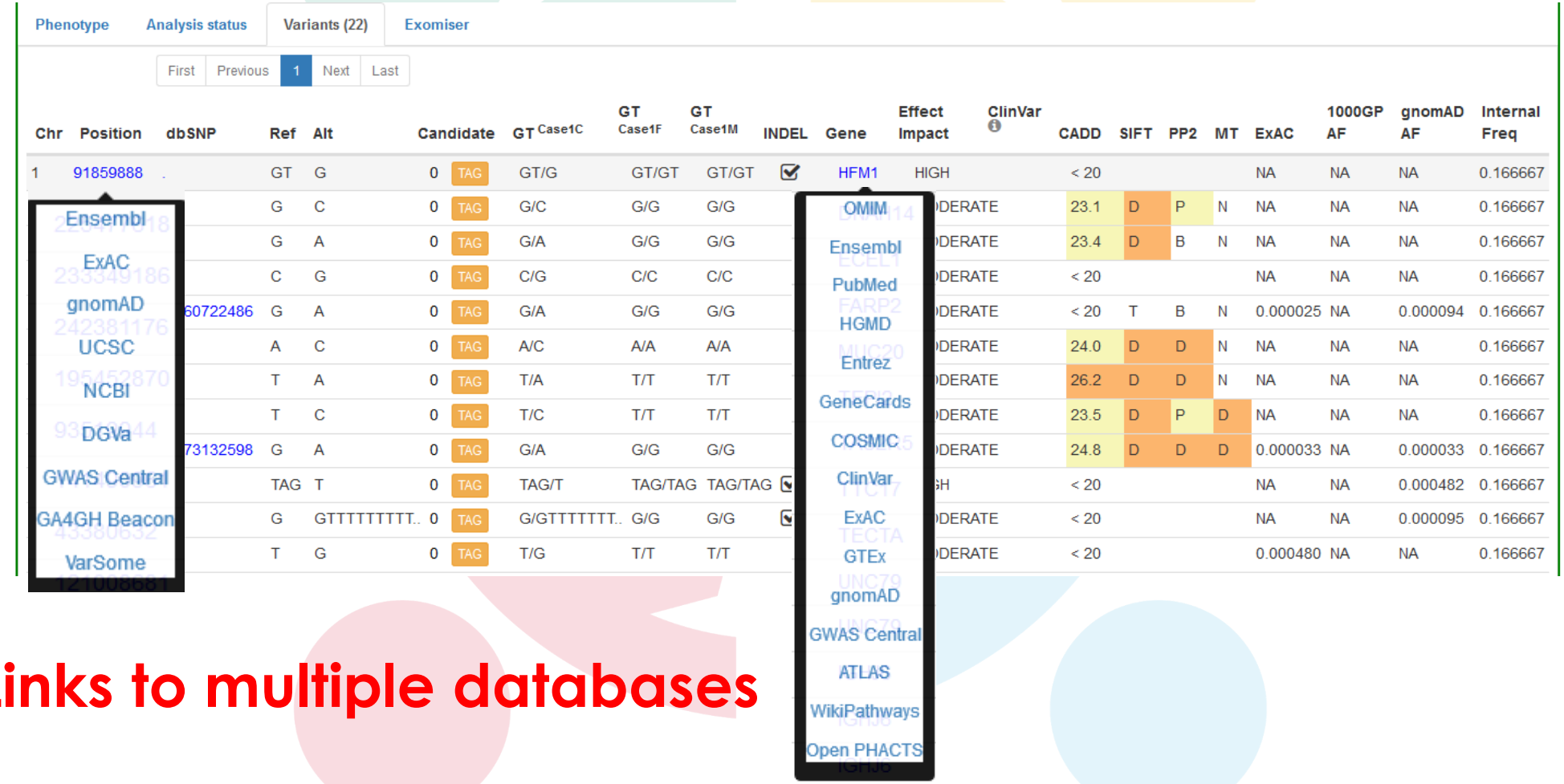
EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

Results section



Links to multiple databases

Funded by the European Union GA n°825575

Results section

e.g direct link to VARSOME – ACMG classification

Verdict

Pathogenic

Transcript NM_006017.2, canonical, protein length 866, gene PROM1, splicing variant

Rules

☑ PVS1 ✔	☒ PS1 ✔	☒ PS2 ✔	☒ PS3 ✔	☐ PS4	☒ PM1 ✔	☑ PM2 ✔	☐ PM3
☒ PM4 ✔	☒ PM5 ✔	☒ PM6 ✔	☒ PP1 ✔	☒ PP2 ✔	☑ PP3 ✔	☐ PP4	☒ PP5 ✔
☒ BA1 ✔	☐ BS1	☒ BS2 ✔	☒ BS3 ✔	☒ BS4 ✔			
☒ BP1 ✔	☐ BP2	☒ BP3 ✔	☒ BP4 ✔	☐ BP5	☒ BP6 ✔	☒ BP7 ✔	

Please tick or untick any rules to switch them on or off - the Verdict will update.

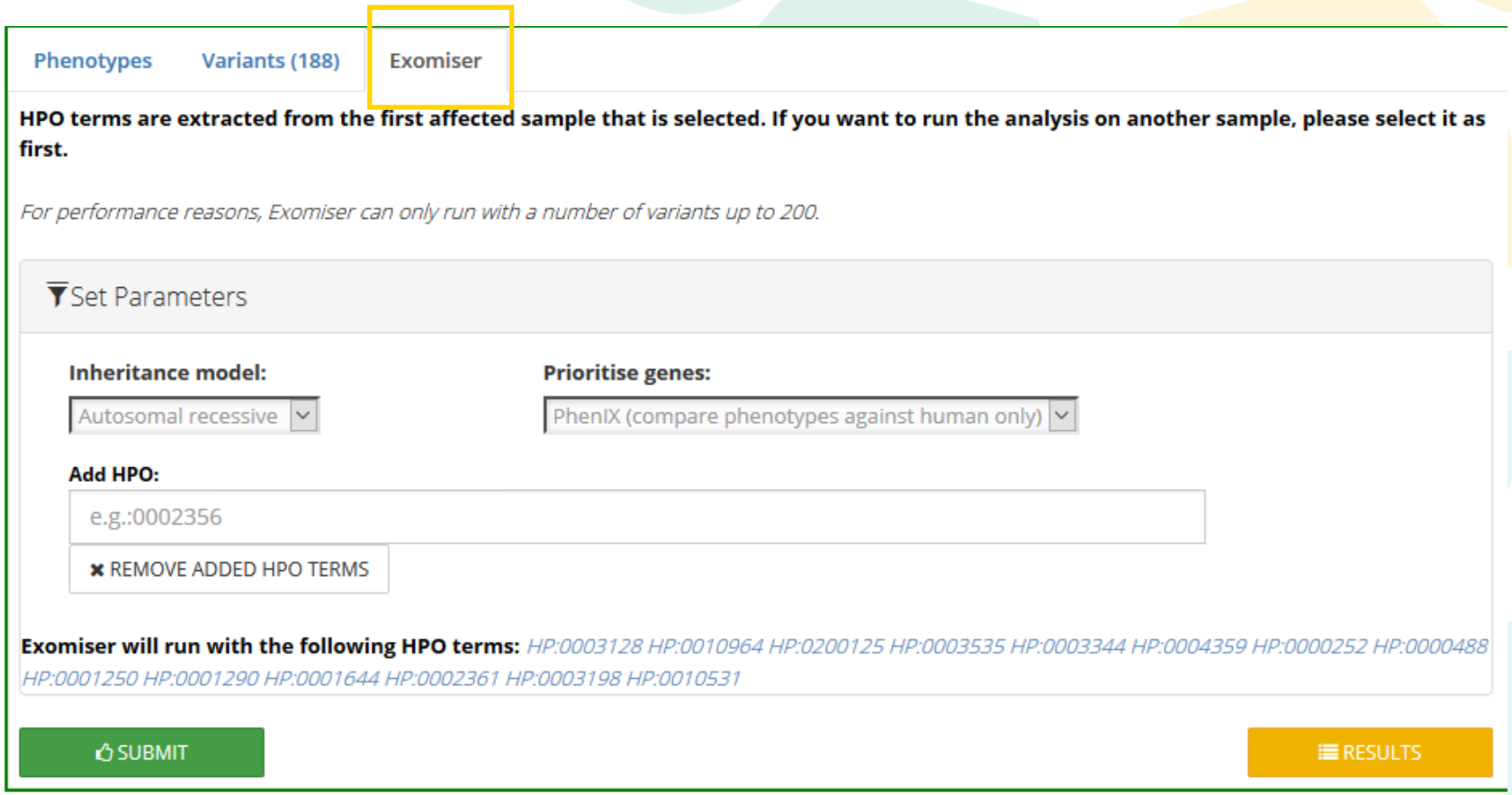
Identified criteria

Rule	Pathogenicity	Explanation
PVS1	Pathogenic Very Strong	Null variant (within ±2 of splice site) affecting gene PROM1, which is a known mechanism of disease (gene has 28 known pathogenic variants which is greater than minimum of 5), associated with Cone-rod dystrophy 12, Macular dystrophy, retinal, 2, Stargardt disease 4 and Retinitis pigmentosa 41.
PM2	Pathogenic Moderate	GnomAD exomes allele frequency = 0.0000161 is less than 0.0001 threshold for recessive gene PROM1 (good GnomAD exomes coverage = 75.1). Variant not found in GnomAD genomes (good GnomAD genomes coverage = 32.6).
PP3	Pathogenic Supporting	Pathogenic computational verdict because 3 pathogenic predictions from DANN, FATHMM-MKL and MutationTaster vs no benign predictions.

Funded by the European Union GA n°825575

Results section

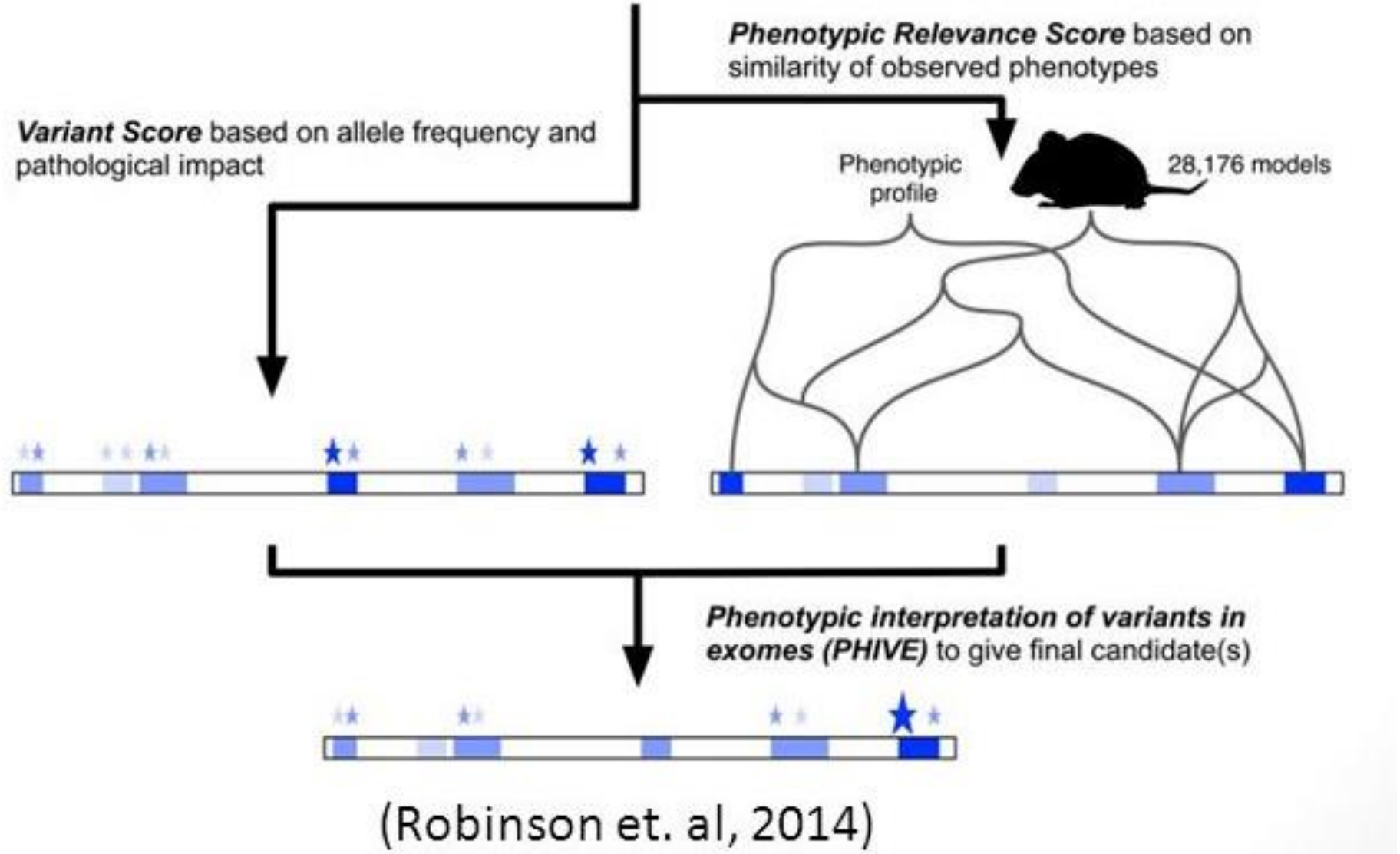
Use Exomiser (IRDiRC recognised resource) to prioritise variants according to patient's HPO terms



Funded by the European Union GA n°825575

Results section

Use Exomiser (IRDiRC recognised resource) to prioritise variants according to patient's HPO terms

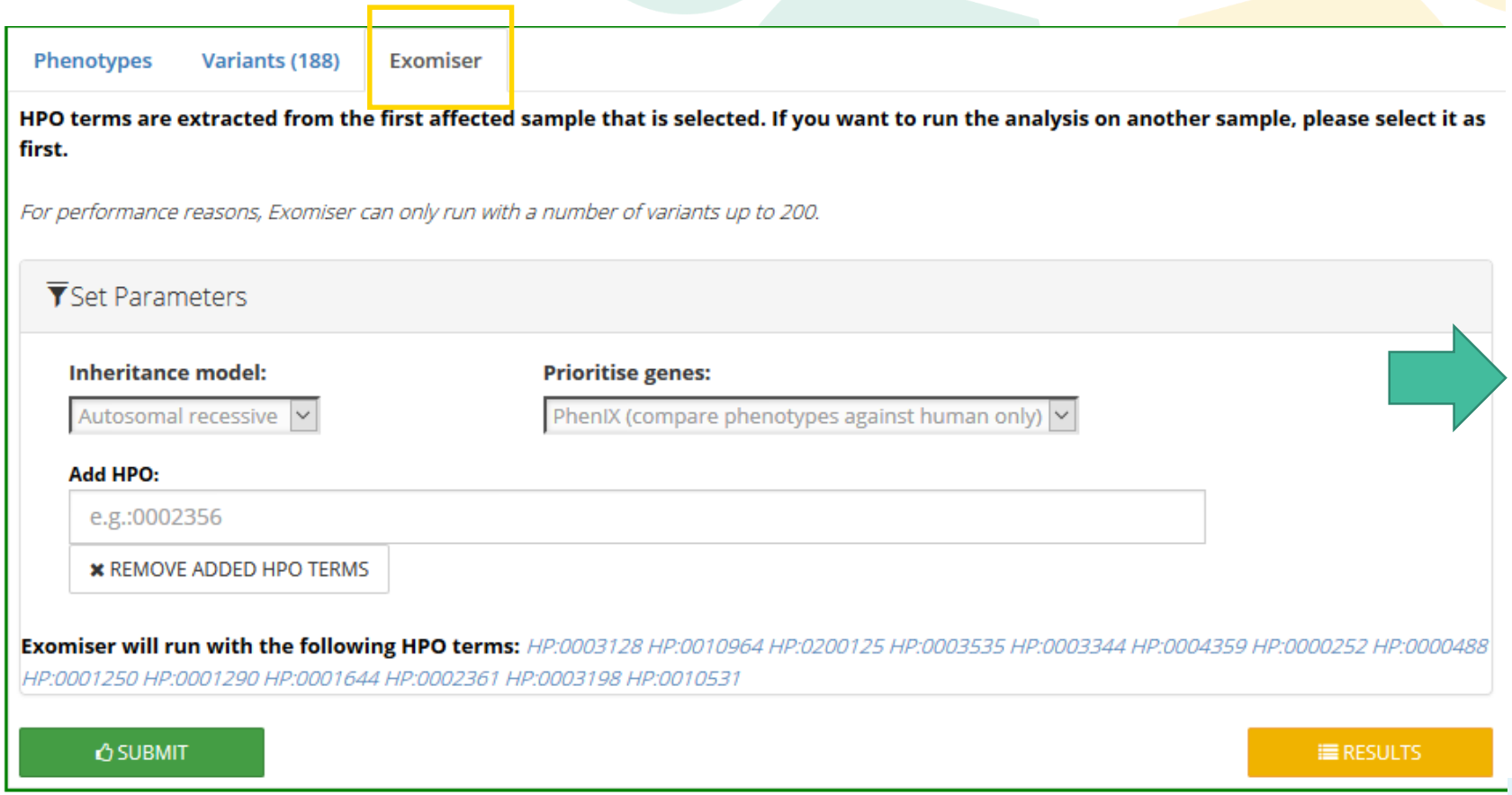


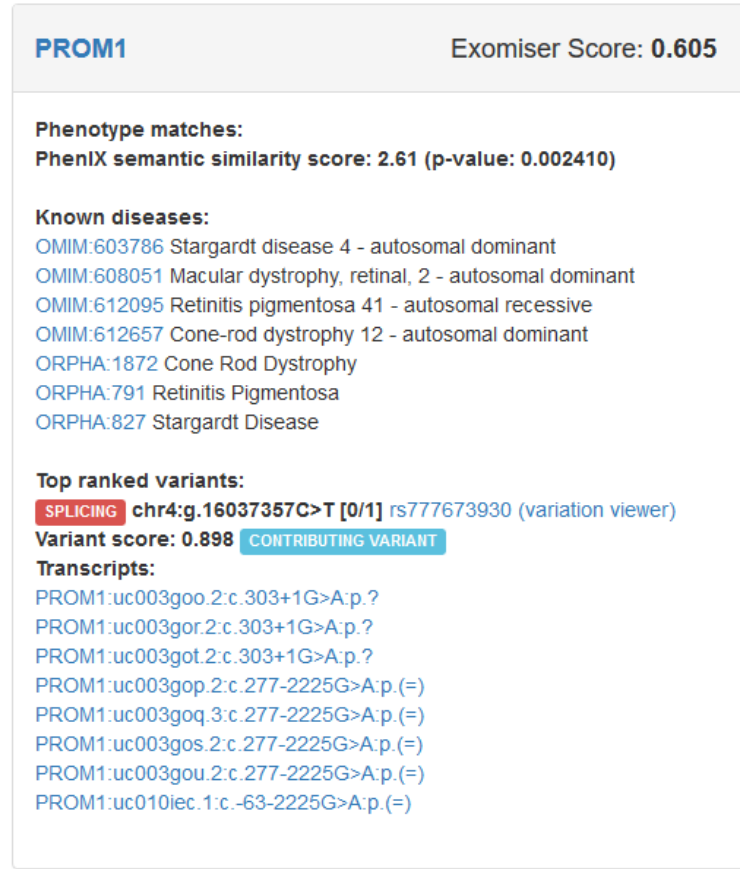
(Robinson et. al, 2014)

Funded by the European Union GA n°825575

Results section

Use Exomiser (IRDiRC recognised resource) to prioritise variants according to patient's HPO terms



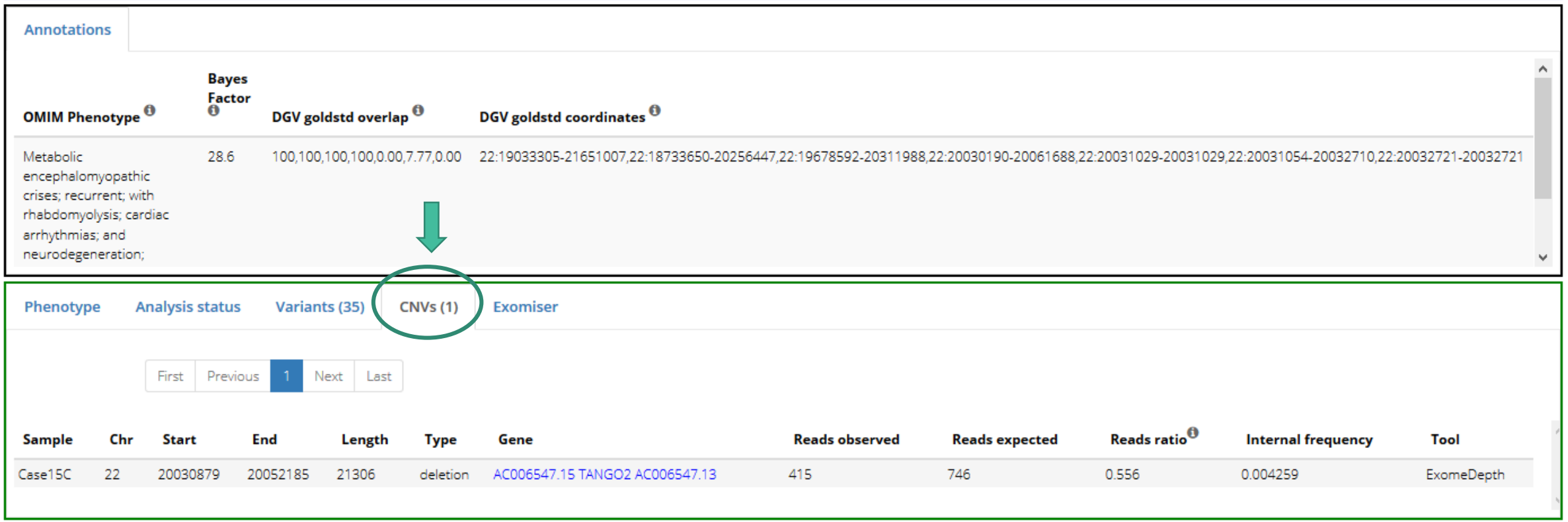


Funded by the European Union GA n°825575

Results section

CNV analysis results available

Multiple tabs with detailed information for data interpretation



Annotations

OMIM Phenotype	Bayes Factor	DGV goldstd overlap	DGV goldstd coordinates
Metabolic encephalomyopathic crises; recurrent; with rhabdomyolysis; cardiac arrhythmias; and neurodegeneration;	28.6	100,100,100,100,0.00,7.77,0.00	22:19033305-21651007,22:18733650-20256447,22:19678592-20311988,22:20030190-20061688,22:20031029-20031029,22:20031054-20032710,22:20032721-20032721

Phenotype | Analysis status | Variants (35) | CNVs (1) | Exomiser

First | Previous | 1 | Next | Last

Sample	Chr	Start	End	Length	Type	Gene	Reads observed	Reads expected	Reads ratio	Internal frequency	Tool
Case15C	22	20030879	20052185	21306	deletion	AC006547.15 TANGO2 AC006547.13	415	746	0.556	0.004259	ExomeDepth

Funded by the European Union GA n°825575

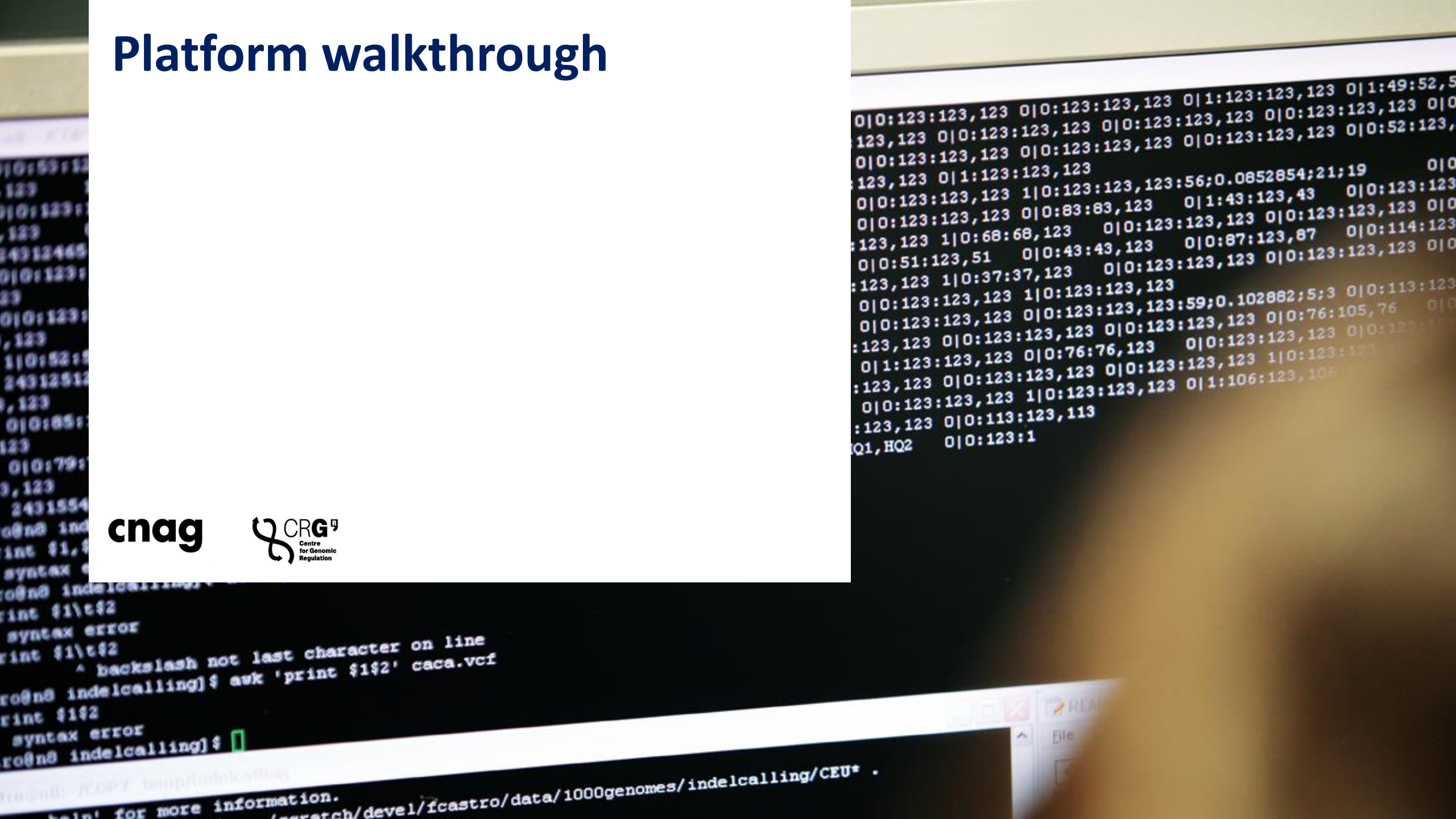
HANDS ON!

Funded by the European Union
GA n°825575

Platform walkthrough

cnag

CRG Centre for Genomic Regulation



Case A: description

https://playground.rd-connect.eu/

RD Connect

Try out the **Genomics Platform** by exploring 50x WGS data for an Illumina Platinum HapMap trio processed using the RD-Connect standard analysis pipeline.

Background

We have generated several trios (father-mother-child), using the same three HapMap samples (NA12877, NA12878, NA12882), but for each trio we have spiked-in real causative variants. A clinical description of these pseudo-cases is provided to download below:

Download phenotypic descriptions

1. Click on Genome-Phenome Analysis Platform and **Login** with username: **test**, password: **1234**
2. Start by going to **"Filters -> Sample Selection -> Select individual Samples"** and add three samples: the individual samples can be selected by typing in **CaseNF**, **CaseNM**, **CaseNC**, but replacing the "N" with the corresponding numbers shown in the phenotypic descriptions file. For example the first trio is **Case1F (father)**, **Case1M (mother)**, **Case1C (child)**.
3. Once you have entered the **IDs**, scroll down to the bottom of the screen and see the phenotypic description of the case (Mode of inheritance, and HPO terms) in the **"Phenotype"** tab.
4. You can apply more **filters** before running the query to try to find the causative variants. **Video tutorials** are available here.

Login

Genome-Phenome Analysis Platform

Log In

Username

test

Password

1234

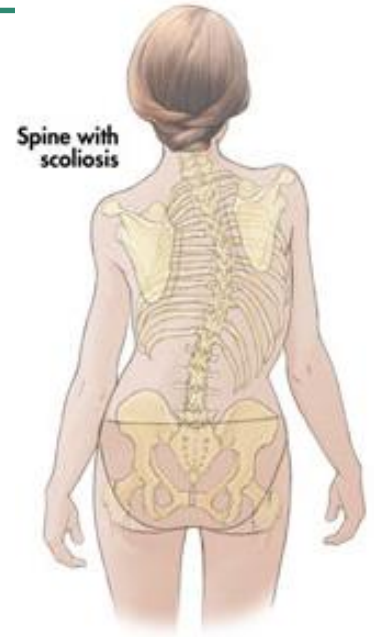
Log In

Funded by the European Union GA n°825575

Case A: description

Indication for referral: congenital myasthenic syndrome
(group of conditions characterised by muscle weakness (myasthenia) that worsens with physical exertion)

Case description: **5 years old girl** that presented with **progressive motor developmental delay**, **mild muscular weakness** in upper legs, **facial hypotonia** and **skeletal abnormalities** involving curving of the spine. **No cognitive impairment**



Family history: First child and no other relatives affected.

PHENOTIPS

EJP RD
cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico
CRG Centre for Genomic Regulation
Funded by the European Union GA n°825575

Case A: description

Patient information

Identifier:

Sex:

Global mode of inheritance:

Sporadic

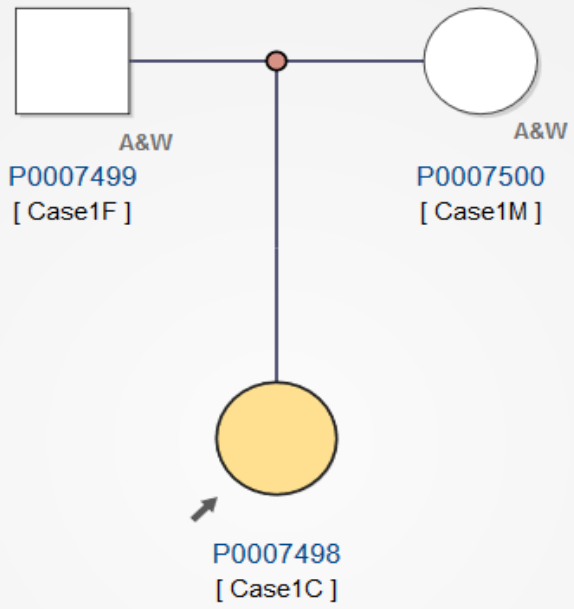
NO **Consanguinity**

Global pace of progression:

Slow progression

Global age of onset:

Congenital onset



Clinical symptoms and physical findings

MUSCLE BULK

Muscle atrophy
Arthrogryposis multiplex congenita
Distal arthrogryposis

WEAKNESS

Neck

MOTOR ABILITY

Inability to walk

can crawl and stand with KAFOs at 4 yrs

LIMBS

Congenital hip dislocation

IMMUNE SYSTEM

Recurrent lower respiratory tract infections

NERVOUS SYSTEM

NO Intellectual disability

MUSCULATURE

Muscular hypotonia
Neonatal hypotonia
Weakness of facial musculature

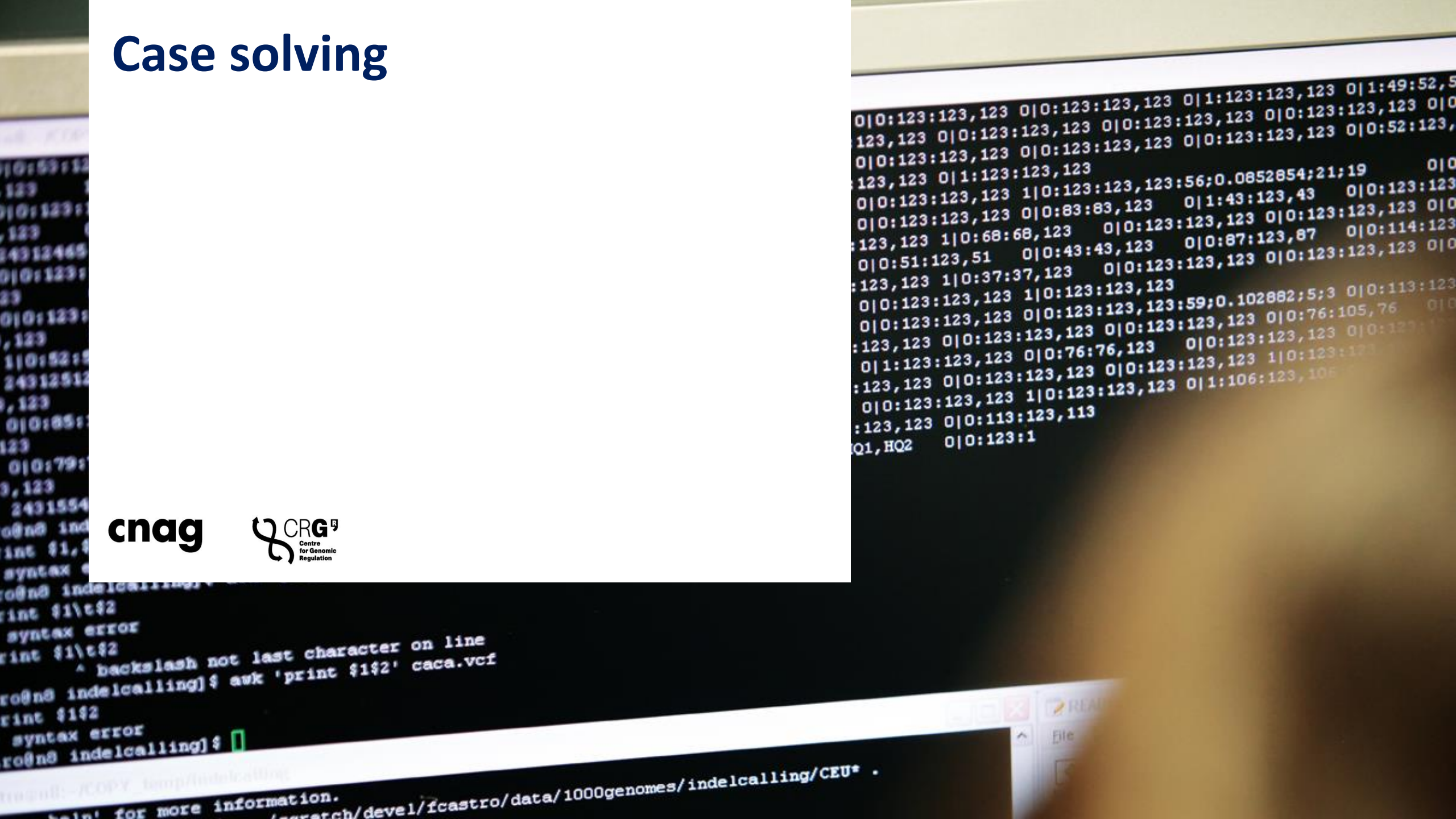
- Pedigree editor
- phenotypic information is entered using **HPO, OMIM and ORDO ontologies**
- **Suspected de novo variant**

Funded by the European Union GA n°825575

Case solving

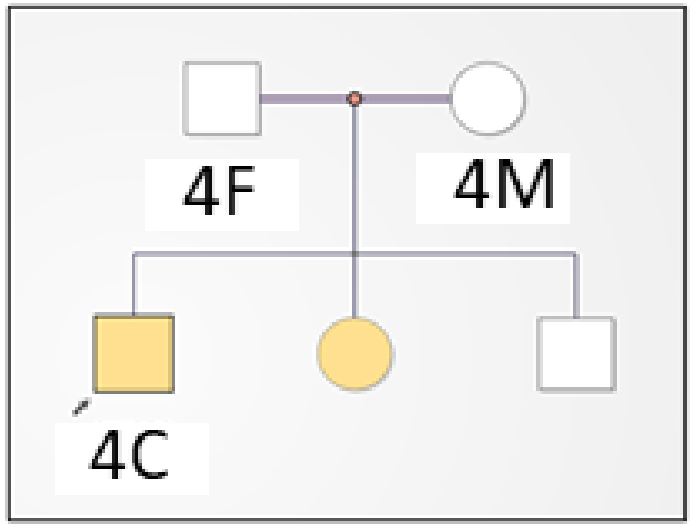
cnag

CRG Centre for Genomic Regulation



Case B: description

Gender	Male
Age	6 years
Referral	Mitochondrial disorder
Onset	Infantile
Global pace of progression	Progressive
Main clinical features	• Microcephaly • Epileptic encephalopathy • Intellectual disability • Motor deterioration

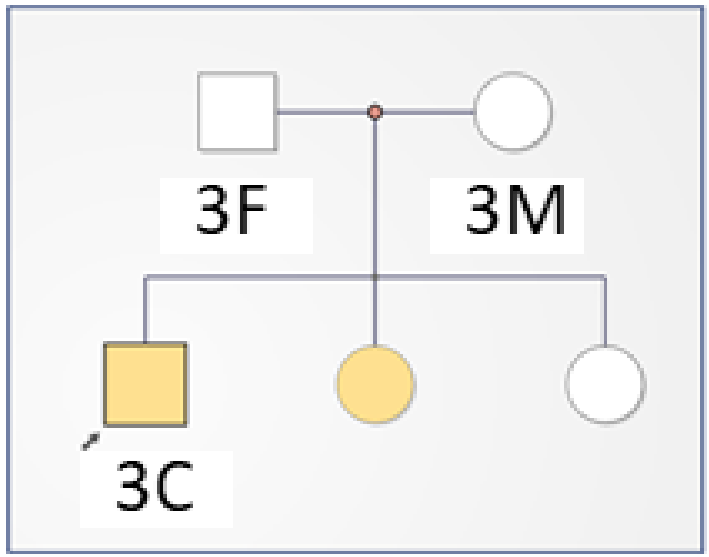


Consanguinity

Funded by the European Union GA n°825575

Case C: description

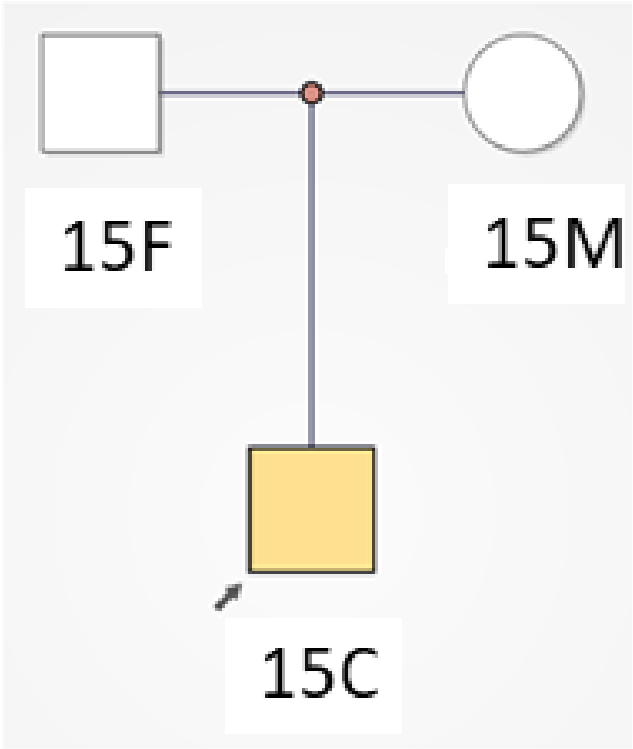
Gender	Male
Age	16 years
Referral	Muscular dystrophy
Onset	Juvenile
Global pace of progression	Progressive
Main clinical features	• Muscle weakness • Dystrophic muscle biopsy • Quadriceps muscle atrophy • Myalgia



Funded by the European Union GA n°825575

Case D: description

Gender	Male
Age	4 years
Referral	Metabolic diseases with epilepsy
Onset	Infantile onset
Global pace of progression	Progressive
Main clinical features	• Rhabdomyolysis • Metabolic acidosis • Seizures • Global developmental delay • Ventricular tachycardia

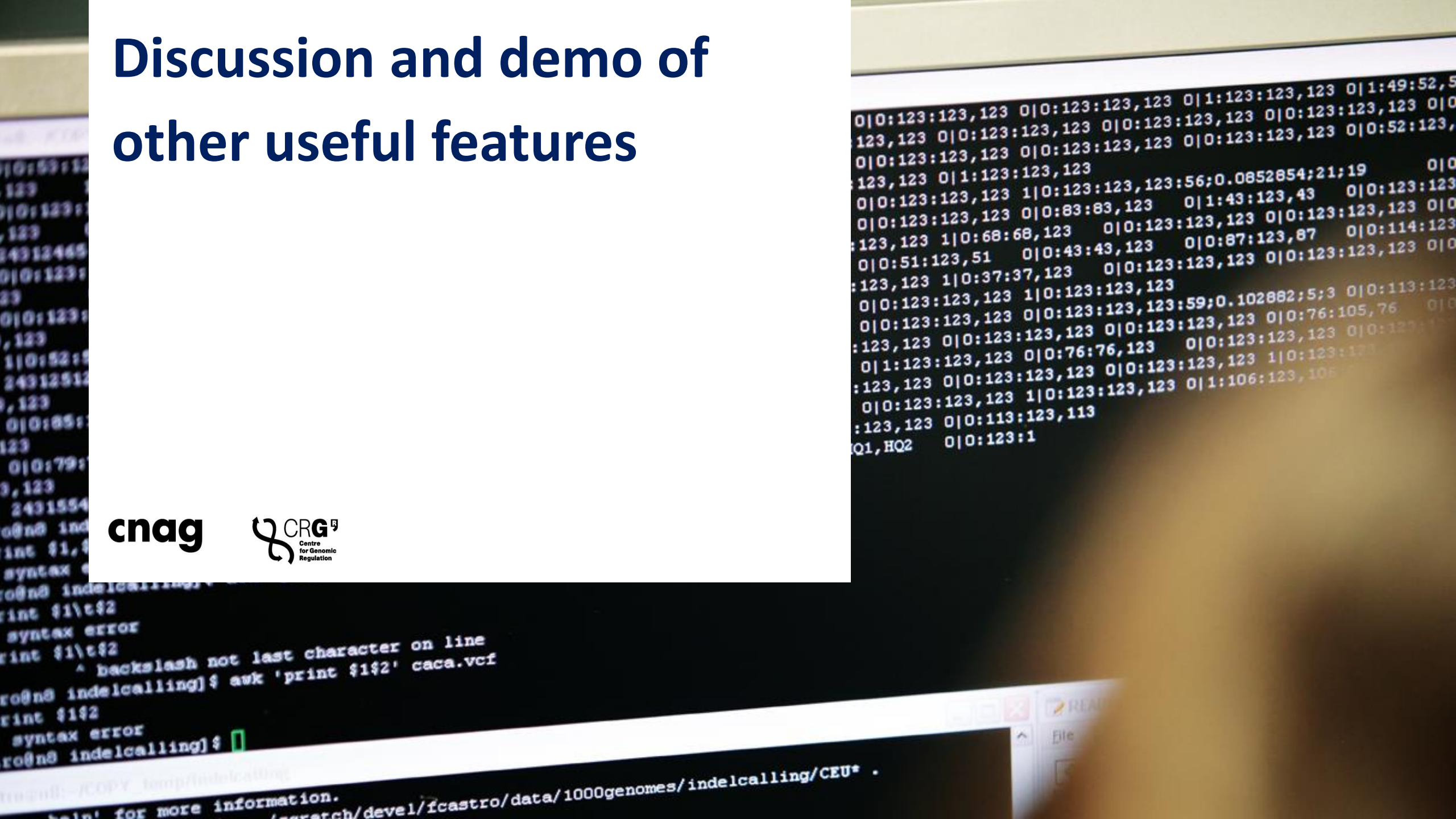


Funded by the European Union GA n°825575

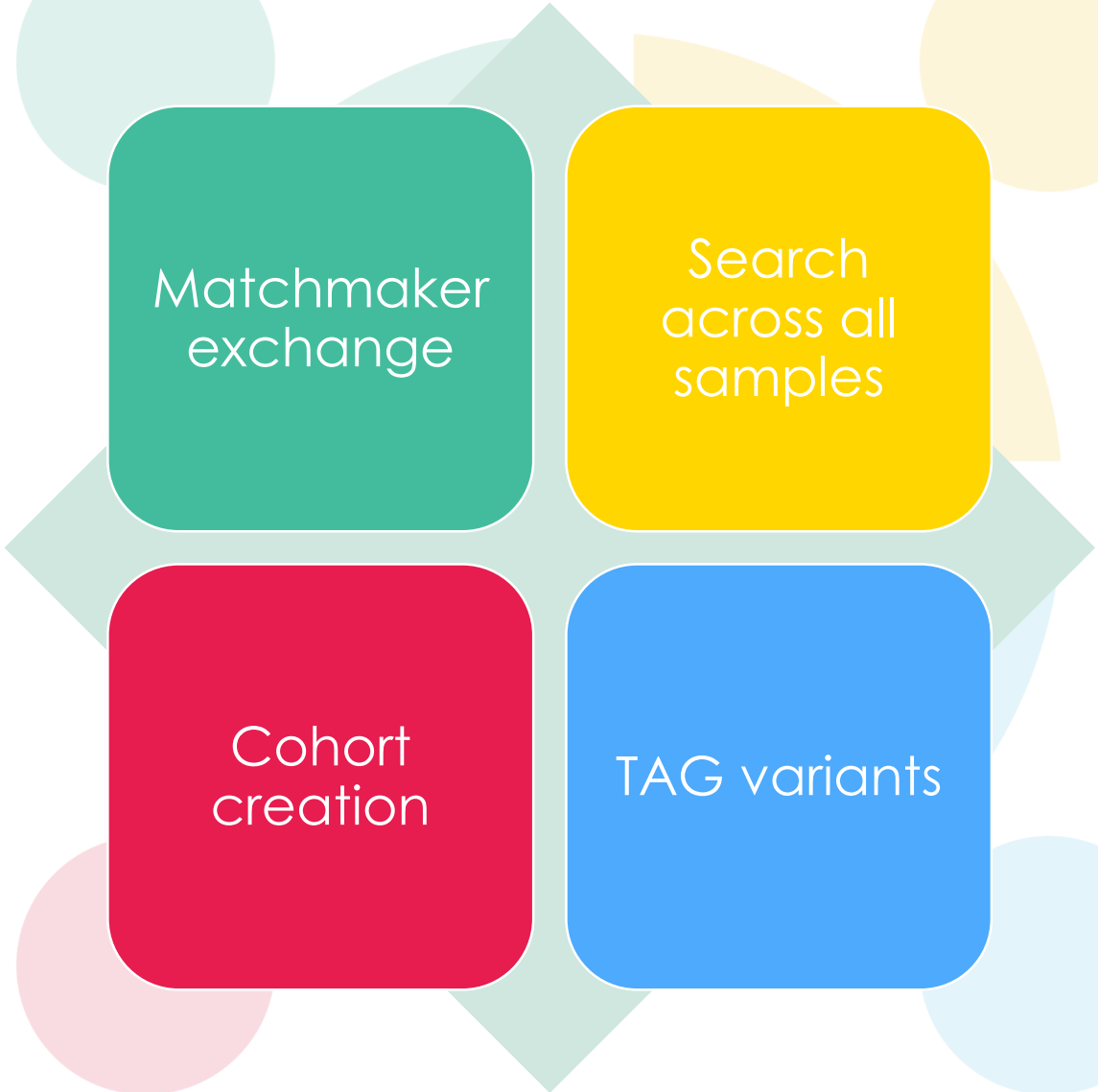
Discussion and demo of other useful features

cnag

CRG Centre for Genomic Regulation



Additional features of interest for data analysis and interpretation



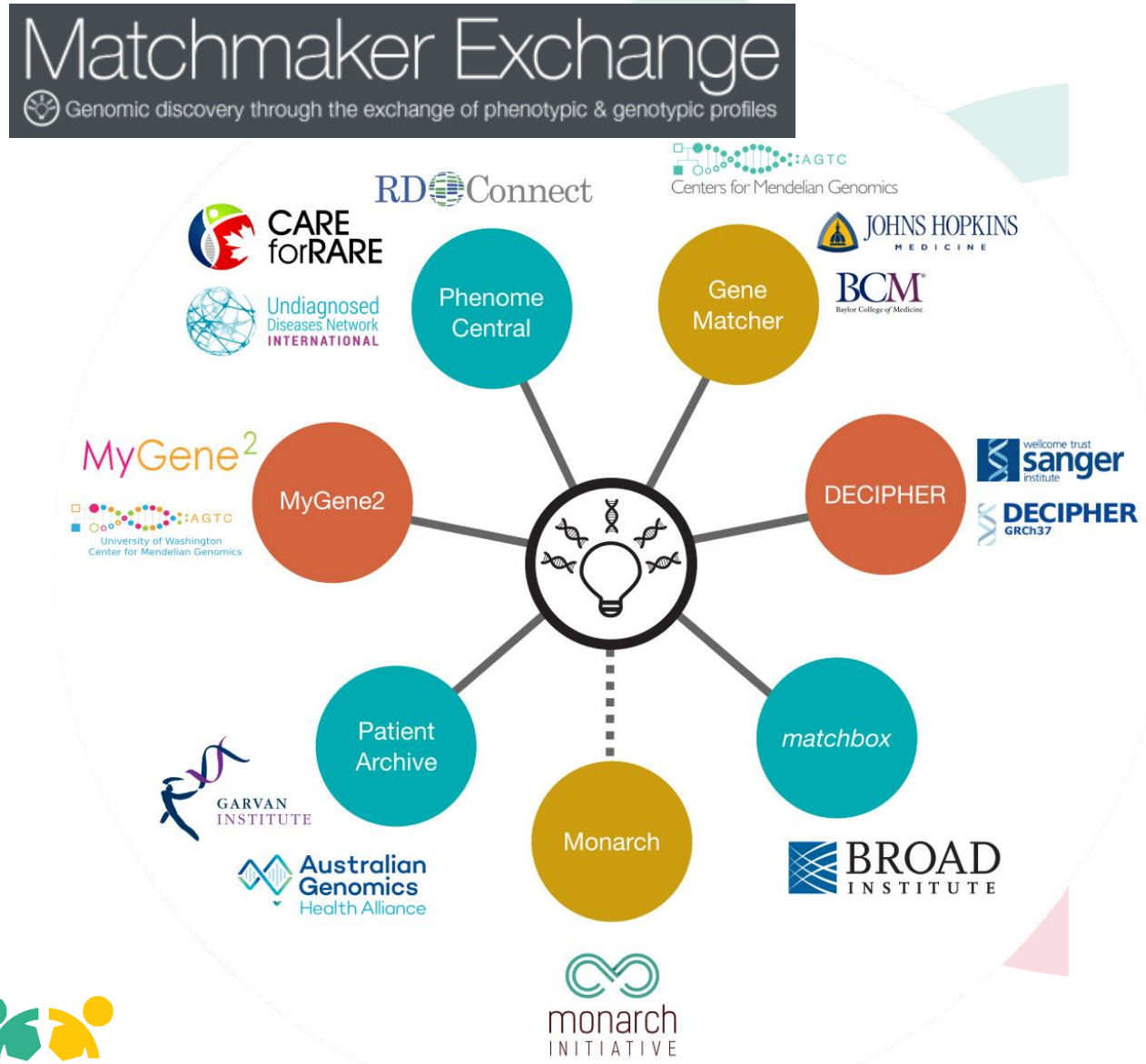
EJP RD

cnag
centre nacional d'anàlisi genòmica
centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union
GA n°825575

Additional features of interest for data analysis and interpretation



Question: Do you have a patient with similar phenotype and genotype as mine?

Human Mutation Volume 36, Issue 10, pages 915-921, 17 SEP 2015 DOI: 10.1002/humu.22858
http://onlinelibrary.wiley.com/doi/10.1002/humu.22858/full#humu22858-fig-0001

Funded by the European Union GA n°825575

Data discovery

Question: Do you have a patient with similar phenotype and genotype as mine?

PhenoTips ID

Target Endpoint

RD-Connect -> RD-Connect

Mode of Inheritance Sporadic **Age of Onset** Neonatal onset

Candidate gene(s)

CHRND

✖ Remove all

Add gene(s)

e.g.:BRCA1

HPO term(s) +

Ptosis,Ophthalmoparesis,Dysphagia,Respiratory insufficiency,Respiratory failure,obsolete Respiratory difficulties,Generalized muscle weakness,EMG: decremental response of compound muscle action potential to repetitive nerve stimulation,Abnormality of muscle morphology

Global Alliance for Genomics & Health
Collaborate. Innovate. Accelerate.

IRDiRC
INTERNATIONAL RARE DISEASES RESEARCH CONSORTIUM

Matchmaker Exchange
Genomic discovery through the exchange of phenotypic & genotypic profiles

Matches found: 16

Score (0 to 1), is based on a gene-match and a phenotypic similarity which is calculated using the: UI score

Contact	Patient	Score	Submitter	Phenotype	Genes
CONTACT		0.72	RD-Connect Matchmaker Exchange	Muscle fiber atrophy ,Abnormality of muscle morphology ,Chewing difficulties ,Abnormality of muscle fibers ,Episodic flaccid weakness ,Proximal muscle weakness ,Type 2 muscle fiber atrophy ,EMG: decre...	CHRND
CONTACT		0.70	RD-Connect Matchmaker Exchange	Fatigable weakness ,EMG: decremental response of compound muscle action potential to repetitive nerve stimulation ,Ptosis ...	CHRND

EJP RD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico

CRG Centre for Genomic Regulation

Funded by the European Union GA n°825575

If you …

- ✓ … would like to submit data to the RD-Connect GPAP
- ✓ … are interested in piloting a local RD-Connect GPAP instance
- ✓ … have questions or would like to explore collaborations

Visit platform.rd-connect.eu

email platform@rd-connect.eu

@ConnectRD

cnag centre nacional d'anàlisi genòmica centro nacional de análisis genómico
CRG Centre for Genomic Regulation
Funded by the European Union GA n°825575

Acknowledgments

Bioinformatics Analysis Unit

Data analysis: S. Beltran, R. Tonda, J.R. Trotta, G. Parra, J. Morata, S. Laurie, L. Matalonga, G. Bullich, I. Paramonov, D. Piscia, A. Papakonstantinou, D. Picó, M. Fernández, A. Corvo, C. Garcia, C. Hernández

Production Bioinformatics: M. Ingham, J. Camps, E. Casals, E. Marmesat

REDES ICTS PerMedOmics

Funded by the European Union GA n°825575