Master 1Bioinformatics

# How to perform genetic diagnosis of rare disorders based on NGS data ?

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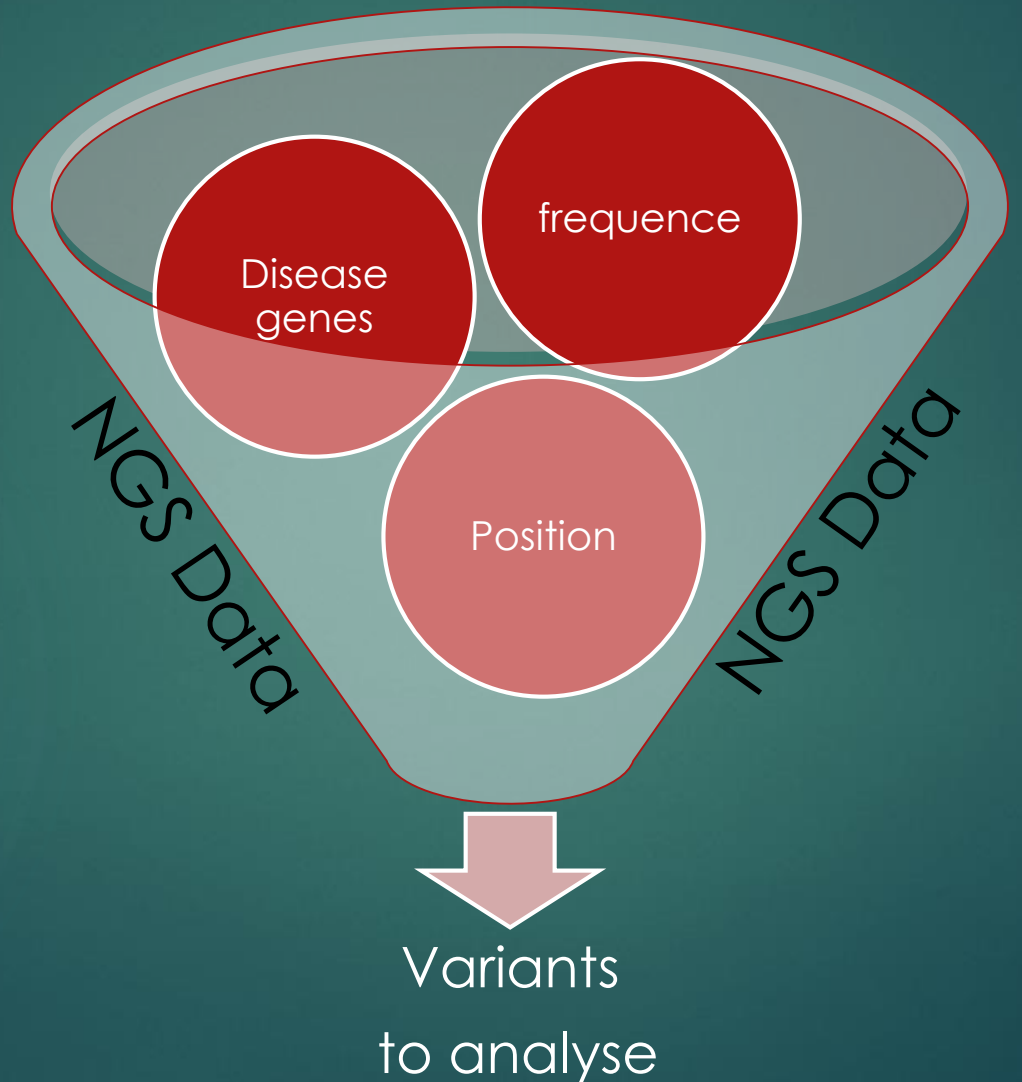
# Rare genetic disorders

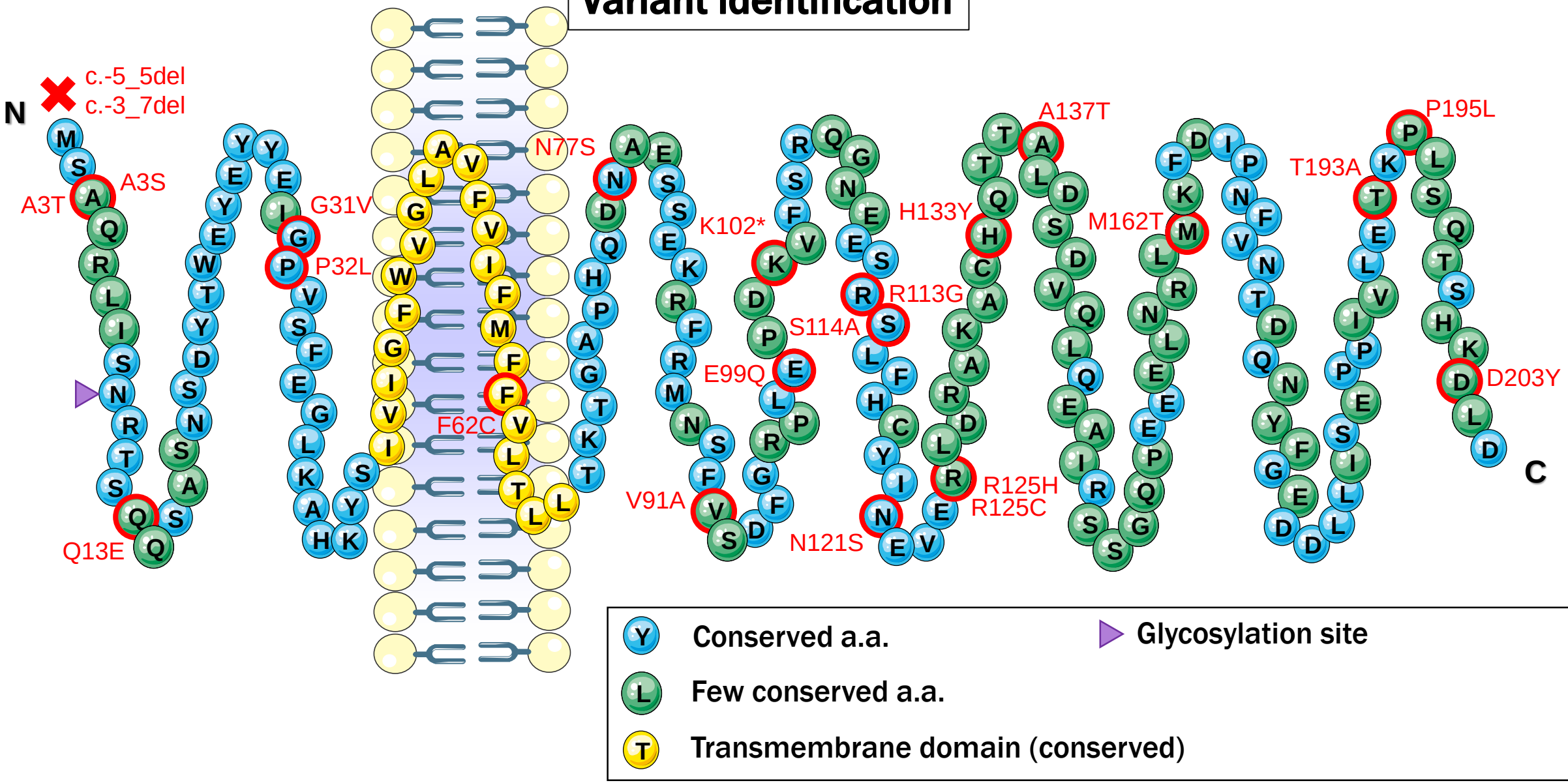
- Genetic disorder = when the illness is caused by one or more abnormalities in the genome
- Rare genetic disorder = when the abnormality is monogenic /located on one gene
- >5000 human diseases are caused by rare genetic disorders

- Only one abnormality can cause the illness!!

# How to filter NGS data ?



# Variant identification

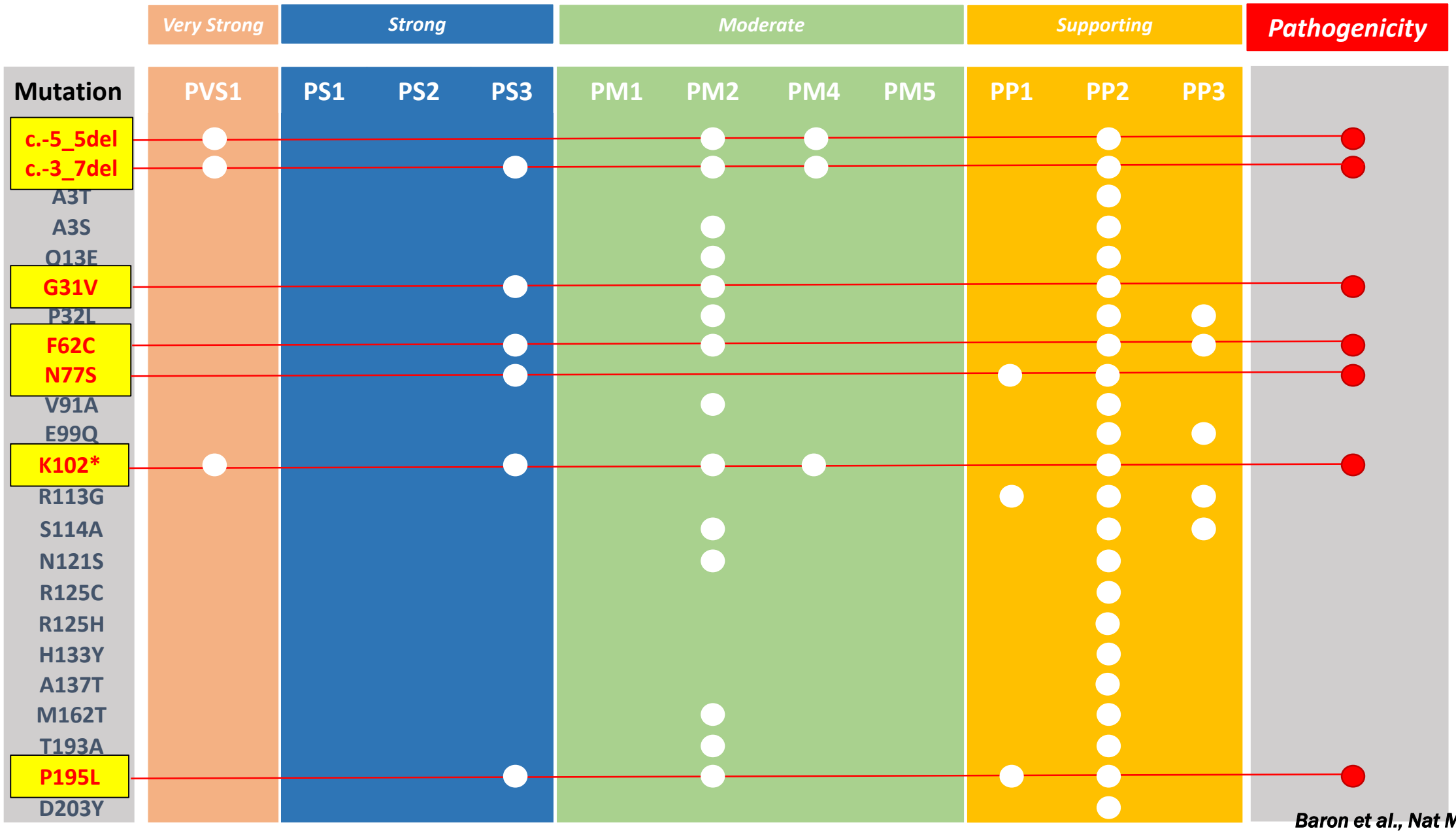



→ 23 mutations have been identified

→ These mutations are statistically associated with obesity and overweight

*Baron et al., Nat Med, 2019*

# Pathogenicity of the variants




Baron et al., Nat Med, 2019

# Diagnosis: ACMG criteria

- ACMG = American College of Medical Genetics and Genomics
- Consensus: criteria for classyfying pathogenic variants

Interest variants


ACMG criteria

Pathogenic P
Likely Pathogenic LP

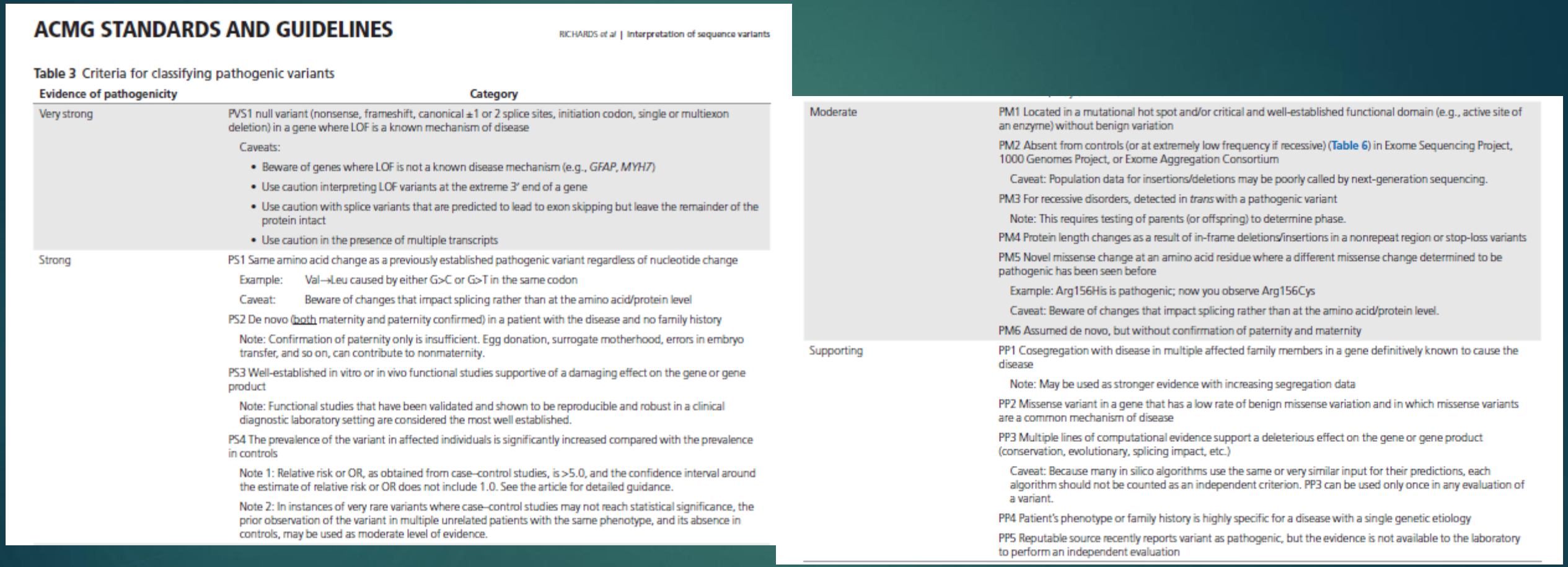
OR

Variant of Uncertain Significance VUS

Causative variant

No diagnosis

# Diagnosis: ACMG criteria



## ACMG STANDARDS AND GUIDELINES



**Table 3** Criteria for classifying pathogenic variants

| Evidence of pathogenicity | Category |
|---|---|
| Very strong | PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease<br>Caveats:<br>• Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*)<br>• Use caution interpreting LOF variants at the extreme 3′ end of a gene<br>• Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact<br>• Use caution in the presence of multiple transcripts |
| Strong | PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change<br>Example: Val→Leu caused by either G>C or G>T in the same codon<br>Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level<br>PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history<br>Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.<br>PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product<br>Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.<br>PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls<br>Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance.<br>Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence. |
| Moderate | PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation<br>PM2 Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium<br>Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.<br>PM3 For recessive disorders, detected in *trans* with a pathogenic variant<br>Note: This requires testing of parents (or offspring) to determine phase.<br>PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants<br>PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before<br>Example: Arg156His is pathogenic; now you observe Arg156Cys<br>Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.<br>PM6 Assumed de novo, but without confirmation of paternity and maternity |
| Supporting | PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease<br>Note: May be used as stronger evidence with increasing segregation data<br>PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease<br>PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)<br>Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.<br>PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology<br>PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation |

**Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology; Genet Med. 2015 May;17(5)**

# Where find data?

- **Databases: HGMD, clinVar, GnomAD….**
- Data about known variants, for example:
- Presence of another variant at the same locus in a gene
- Allelic frequence in general populations
- Evidence of deleterious effect on the gene or on the gene product
- Scientific publication about mutations
- In vivo functionnal studies

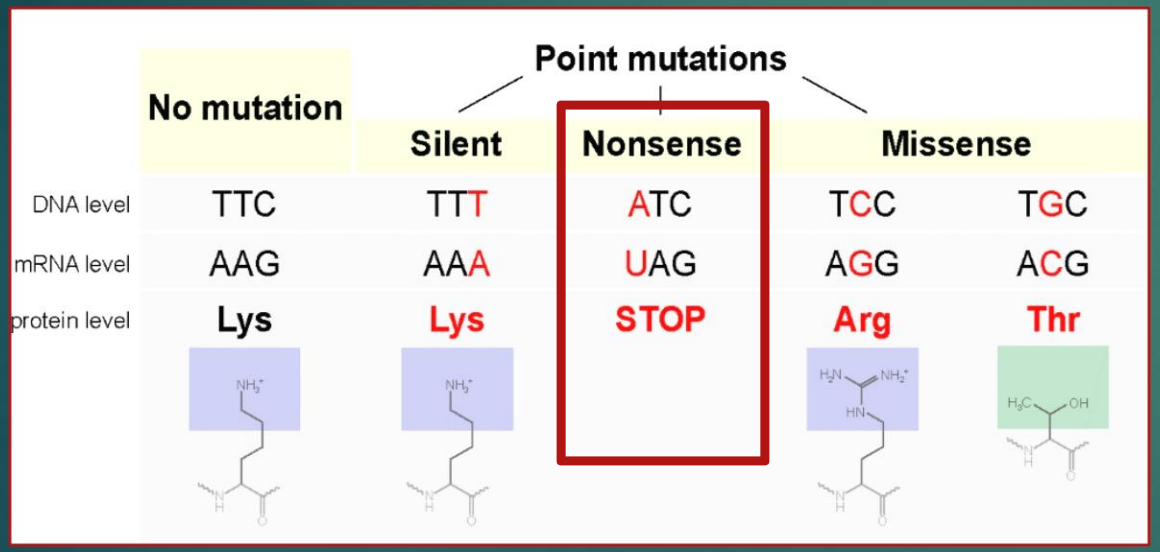
- **Alamut software** is a convenient access to several databases of known variants
- Warning: always check that you explore the same transcript : NM_.....

- **Prediction algorhytmes:**
- Splice site prediction
- Nucleotide conservation prediction

All these data are needed in order to classify the variants

# PVS1 criterion (Pathogenicity Very Strong)

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion




Nonsense mutation: check the Amino Acid change: *=stop

Examples:

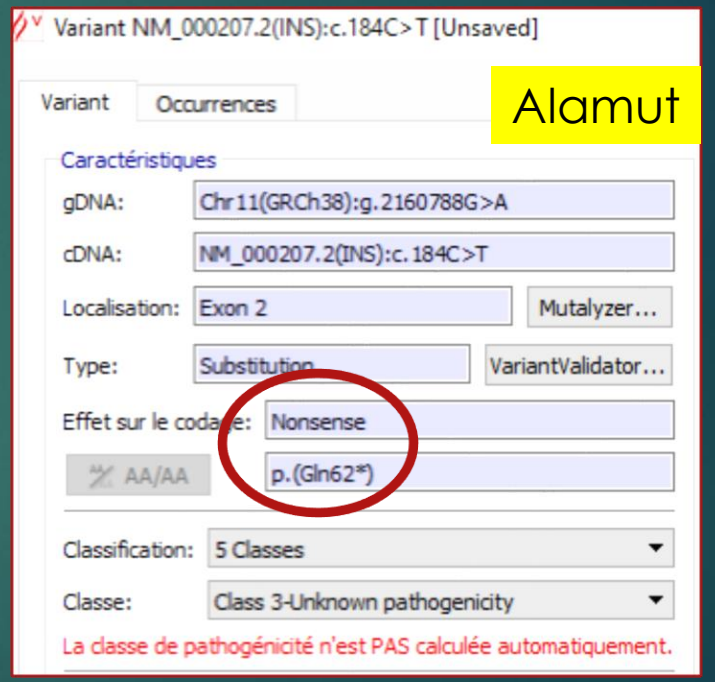
| | | |
|---|---|---|
| NM_000207.2 | c.184C>T, | p.(Gln62*) |
| NM_000207.2 | c.324C>G, | p.(Tyr108*) |
| | cDNA | protein |

# PVS1 criterion

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion

Nonsense mutation: check the Amino Acid change: *=stop
Exemples:
gene INS (AD)
NM_000207.2 c.184C>T, p.(Gln62*) ht, AD
NM_000207.2 c.324C>G, p.(Tyr108*) ht, AD

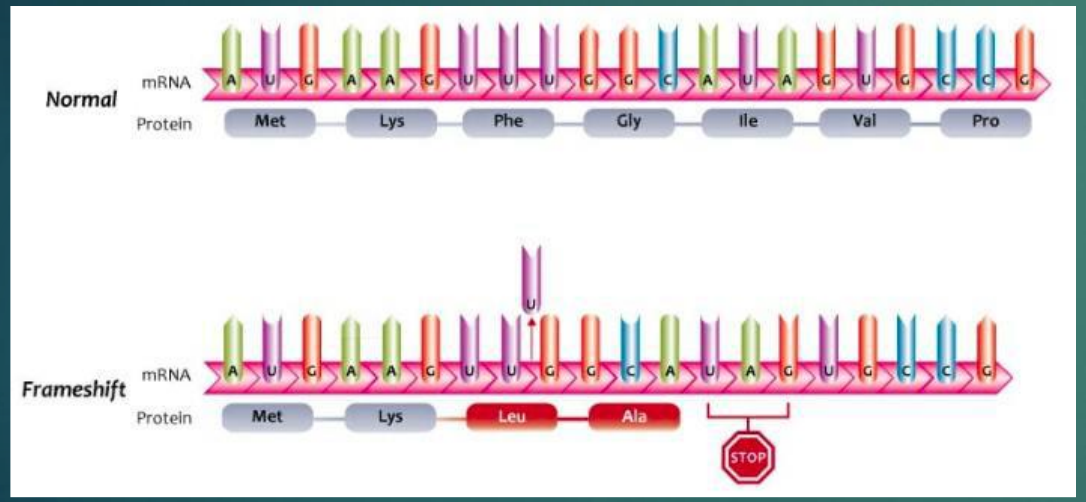
cDNA protein




Where to check:
- NGS Annotation File
- Alamut software
- Public databases

# PVS1 criterion

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion




Frameshift mutation: deletion/insertion in which the number of deleted base pairs is not divisible by three:
check the Amino Acid change and consequences
Example:
c.2711-2714del // p.(His905Alafs*34)

Warning: Indel of multiple of 3 nucleotides = indel of amino acid without frameshift

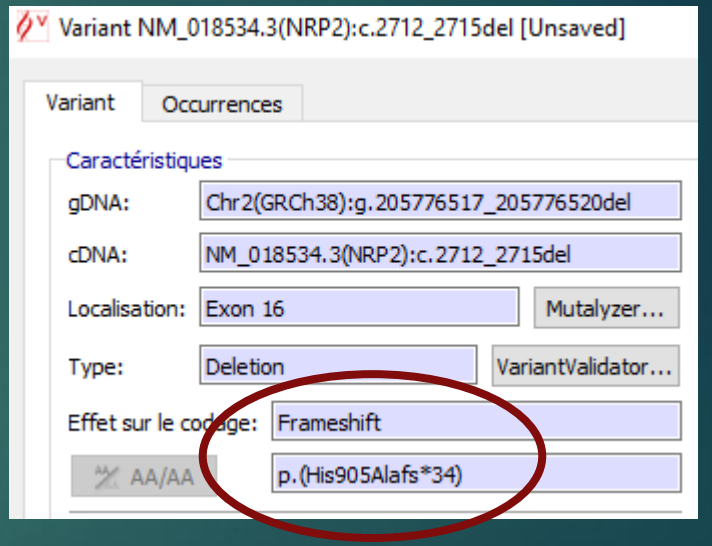
# PVS1 criterion

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion

Alamut

Frameshift mutation: in case of insertion or deletion: check the Amino Acid change and consequences
Exemple:
NM_018534 c.2712-2715del // p.(His905Alafs*34)

Warning: Insertion /deletion of multiple of 3 nucleotides = indel of amino acid without frameshift



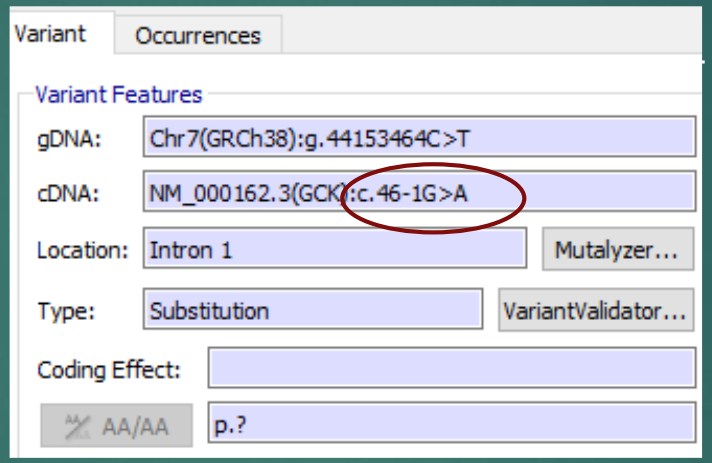

Where to check:
- NGS Annotation File
- Alamut software
- Public databases

# PVS1 criterion

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion

Splice site variant: at the boundary of an exon and an intron .
For example: gene GCK (AD)
Intronic:
NM_000162.3 c.46-4G>A, ht

Exonic:
NM_000162.3 c.211G>C , ht
(intron start after c.211)



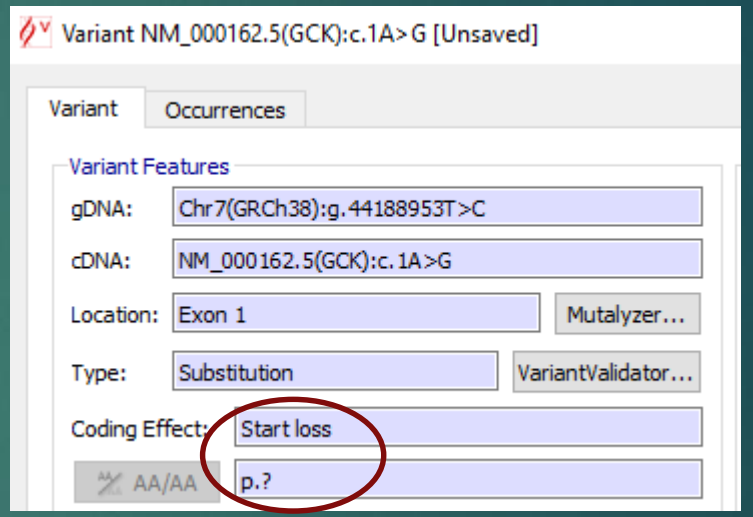

Alamut


Where to check:
- Alamut software
- Splicing prediction tools

# PVS1 criterion

- null variant = nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion

Initiation codon: first ATG = Methionine = M
exemple: c.1A>G, p.?




Alamut

single or multi-exon deletion

# PS1 criterion (Pathogenicity Strong)

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

example: Val -> Leu caused by either G > C or G > T in the same codon


GTG

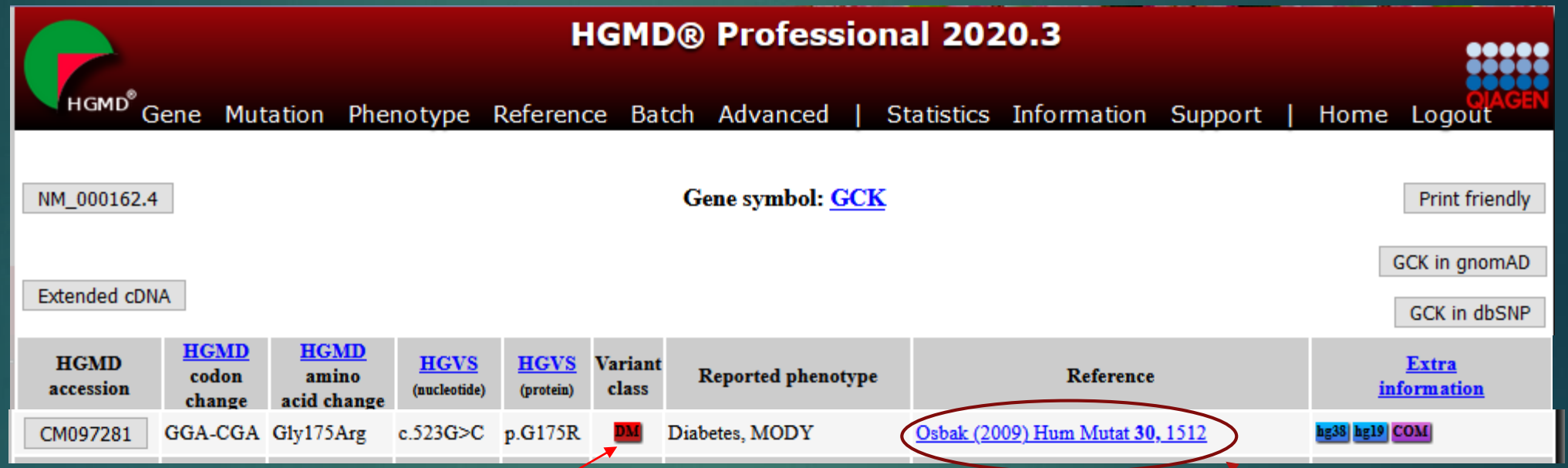
CTG Pathogenic variant present in databases

TTG Interest Variant

Can been checked in Alamut (database ClinVar ) and/or in HGMD

# PS1 criterion (Pathogenicity Strong)

HGMD




DM =Disease Mutation
**DM= PS1!** (read publication)

Publication

Variant: NM_000162.5(GCK):c.523G>C // p.Gly175Arg
Already present in HGMD (in this case with the same nucleotide change)

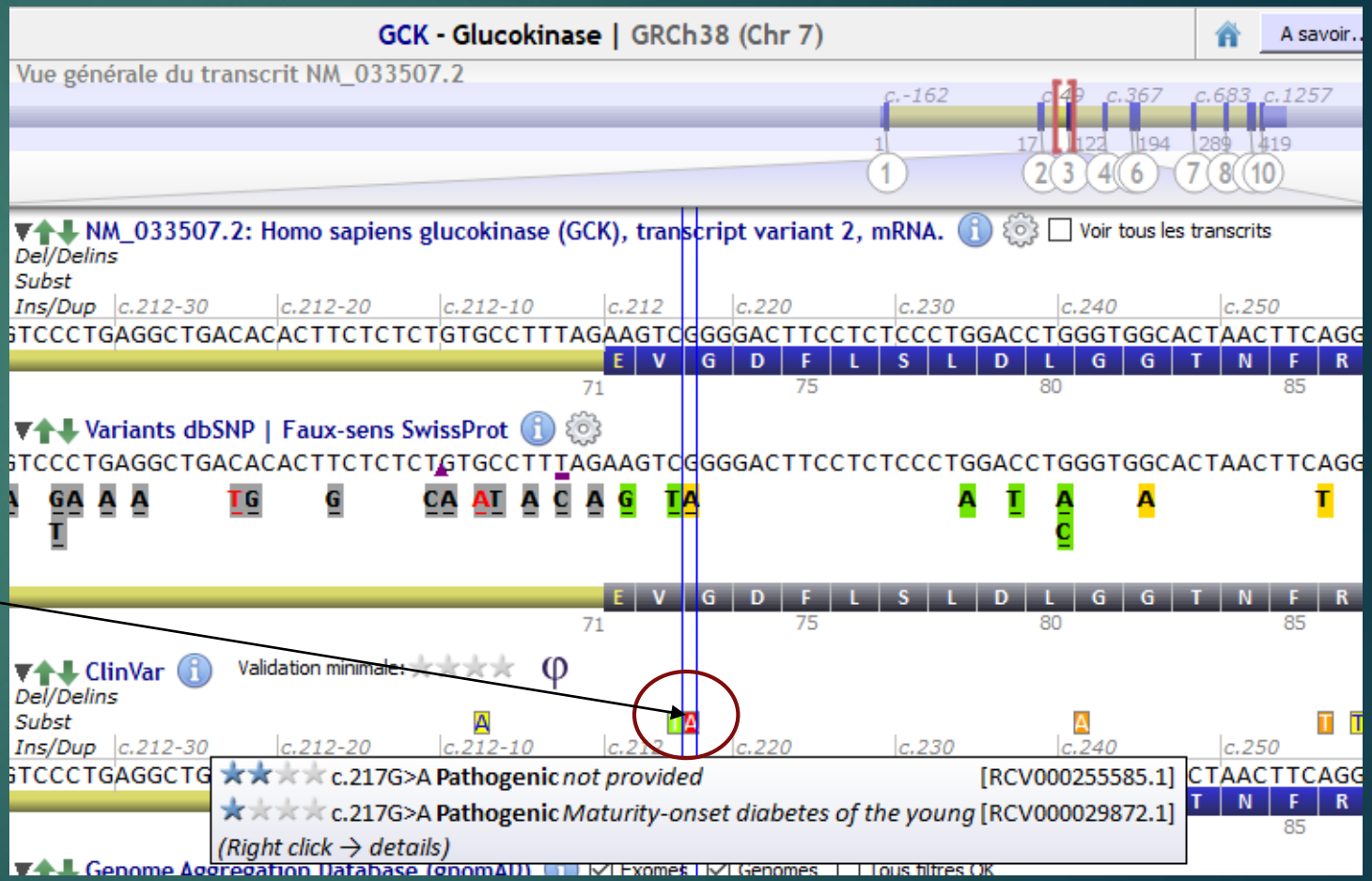
In HGMD, check by categories: Missense/nonsense, splicing mutations, insertions, deletions

# PS1 criterion (Pathogenicity Strong)

ClinVar
via Alamut

Variant:
Gene GCK (AD/AR)
NM_000162.5(GCK):c.217G>A,
p.(Gly73Arg), ht

Red =pathogenic
Orange = likely pathogenic
Green, uncertain significance
**Red = PS1! (read publication)**



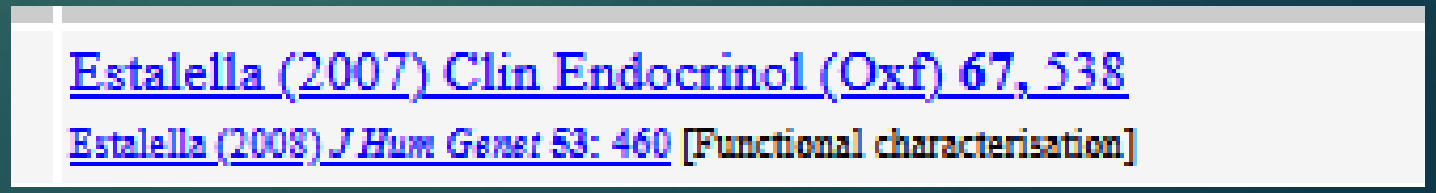
# PS3 criterion (Pathogenicity Strong)

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product

HGMD

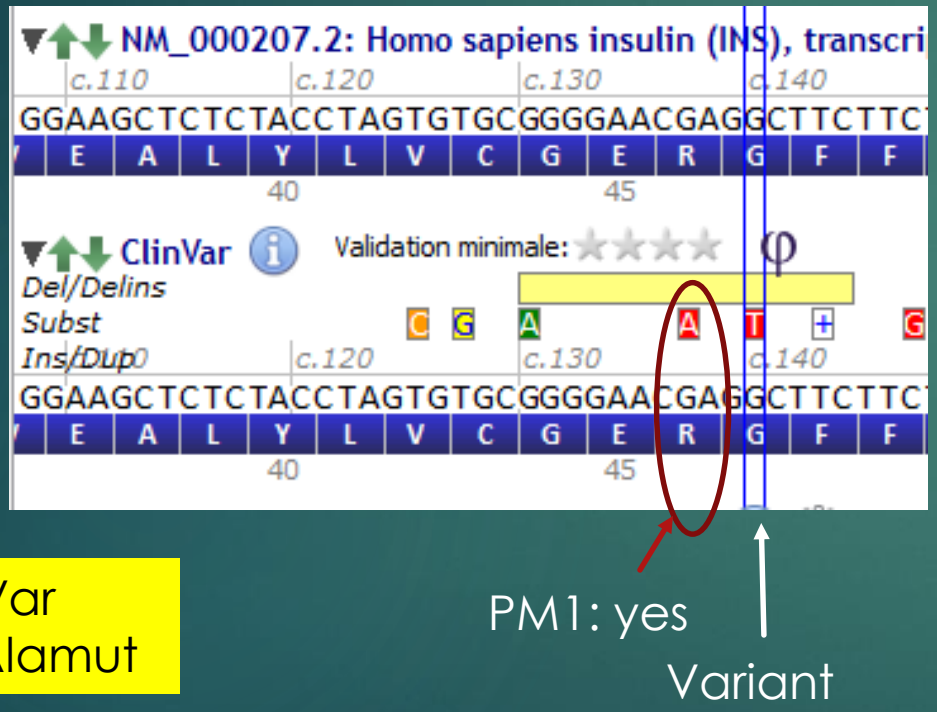

DM =Disease Mutation

**reference with [Functional characterisation]= PS3!** (read publication)



# PM1 criterion (Pathogenicity Moderate)

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation




ClinVar via Alamut

PM1: yes

Variant

Hotspot: pathogenic variant (red) in one residue before or after the interest variant without benign variant (green)

# PM1 criterion (Pathogenicity Moderate)

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation

HGMD

| HGMD accession | HGMD codon change | HGMD amino acid change | HGVS (nucleotide) | HGVS (protein) | Variant class | Reported phenotype | Reference | Extra information |
|---|---|---|---|---|---|---|---|---|
| CM1812672 | TTA-TCA | Leu662Ser | c.1985T>C | p.L662S | DM | Obesity | Kleinendorst (2018) J Med Genet 55, 578<br>Kleinendorst (2017) *BMJ Case Rep* 2017: [Additional report] | hg38 hg19 dbSNP |
| CM070187 | CAT-CCT | His684Pro | c.2051A>C | p.H684P | DM | Obesity, early-onset | Farooqi (2007) N Engl J Med 356, 237<br>Kimber (2008) *Endocrinology* 149: 6043 [Functional characterisation]<br>Clément (2018) *Nat Med* 24: 551 [Additional case report]<br>2 more reference(s)... | hg38 hg19 dbSNP gnomAD |
| CM168926 | TCT-TTT | Ser723Phe | c.2168C>T | p.S723F | DM | Obesity, severe | Hannema (2016) Horm Res Paediatr 85, 412<br>Kleinendorst (2017) *BMJ Case Rep* 2017: [Additional report]<br>Kleinendorst (2018) *J Med Genet* 55: 578 [Additional report] | hg38 hg19 |

Example:
NM_002303.5(LEPR):c.2047C>T, p.(His683Tyr), ht

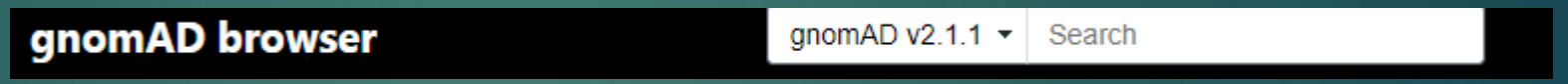
In case of misense or insertion or deletion, check missense variants

Hotspot: pathogenic variant DM in one residue before or after the interest variant without benign variant (green)

# PM2 criterion (Pathogenicity Moderate)

PM2 Absent from controls (or at extremely low frequency if recessive) in GnomAD

Where to check?




| Variant ID | Source | Consequence | Annotation | Flags | Allele Count | Allele Number | Allele Frequency | Number of Homozygotes |
|---|---|---|---|---|---|---|---|---|
| 11-2181011-T-C | E G | c.*71A>G | 3' UTR | | 3 | 236228 | 1.27e-5 | 0 |
| 11-2181016-C-T | E | p.Glu92Lys † | missense | | 1 | 211970 | 4.72e-6 | 0 |
| 11-2181023-T-C | E | p.Lys89Lys † | synonymous | | 1 | 220918 | 4.53e-6 | 0 |
| 11-2181028-T-C | G | p.Asn88Asp † | missense | | 1 | 31316 | 3.19e-5 | 0 |
| 11-2181029-C-T | E | p.Trp87Ter † | stop gained | LC pLoF pLoF flag | 1 | 226462 | 4.42e-6 | 0 |
| 11-2181031-ATC-A | G | p.Arg86MetfsTer3 † | frameshift | LC pLoF pLoF flag | 1 | 31194 | 3.21e-5 | 0 |
| 11-2181037-C-G | E | p.Glu85Gln † | missense | | 7 | 232958 | 3e-5 | 0 |

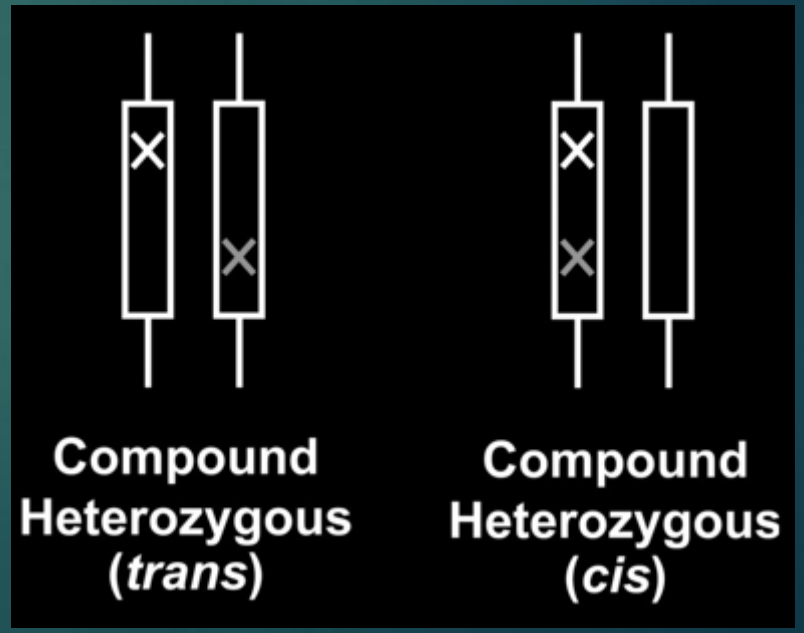
Example: NM_000207.2(INS): p.(Ile91Val), ht: PM2=yes
NM_000207.2(INS): p.(Glu92Lys), ht: PM2=no, homoz:PM2= yes

# PM3 criterion (Pathogenicity Moderate)

- PM3 For recessive disorders, detected in *trans* with a pathogenic variant
- Note: This requires testing of parents (or offspring) to determine phase.

Two pathogenic variants in the same gene:

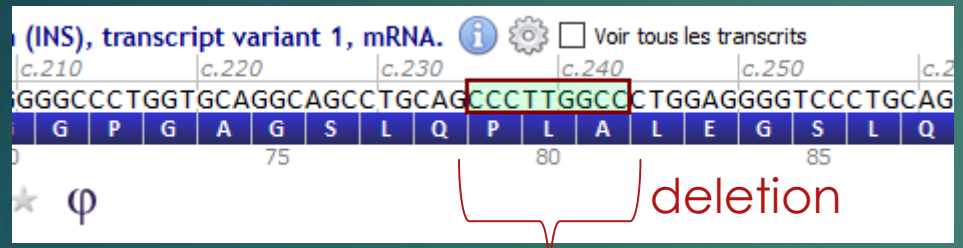
Sequencing of the parents:
- one parent is carrier of the two variants: PM3 = no
- each parent is carrier of one variant: PM3 = yes

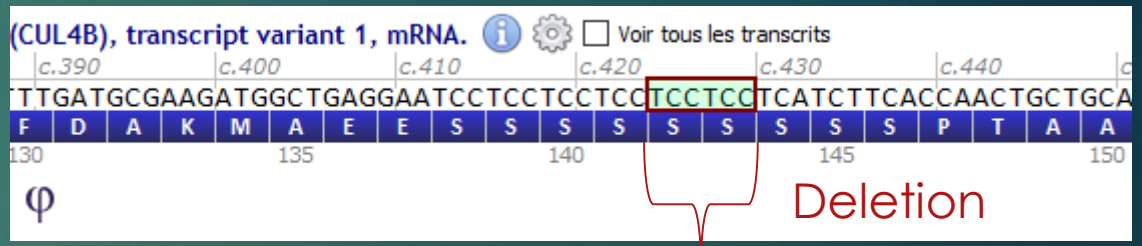


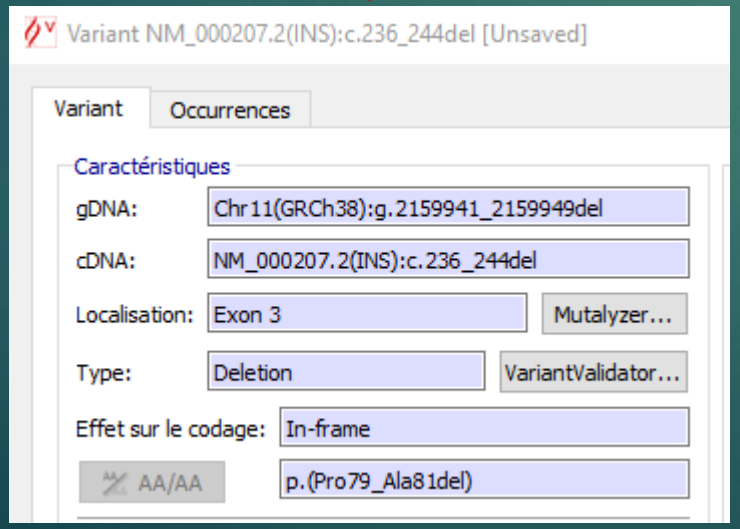
# PM4 criterion (Pathogenicity Moderate)

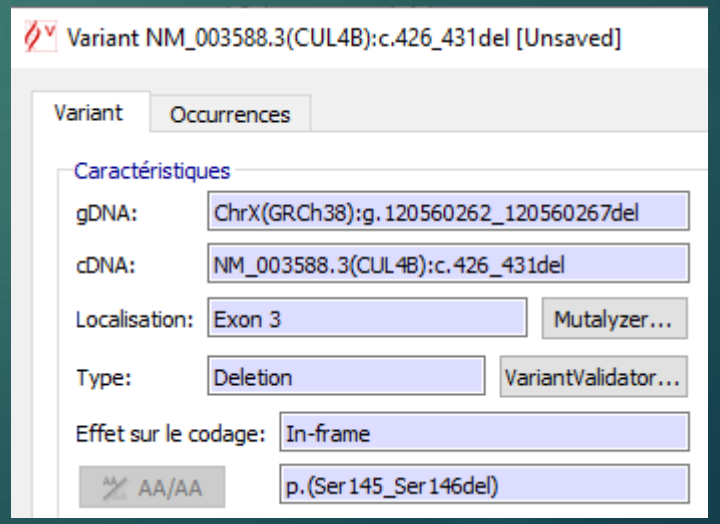
- PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants

Alamut




PM4: yes

PM4: no

# PM5 criterion (Pathogenicity Moderate)

- Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
- Example: Arg156His is pathogenic; now you observe Arg156Cys

- NM_000162.5(GCK):c.67T>C, p.(Phe23Leu)

HGMD

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CM191975 | CTG-CGG | Leu20Arg | c.59T>G | p.L20R | DM | Diabetes, gestational | Zubkova (2019) Acta Diabetol , |
| CM074228 | CTG-CCG | Leu20Pro | c.59T>C | p.L20P | DM | Diabetes, MODY | Estalella (2007) Clin Endocrinol (Oxf) **67**, 538 |
| CM096803 | TTC-GTC | Phe23Val | c.67T>G | p.F23V | DM | Diabetes, MODY | Osbak (2009) Hum Mutat **30**, 1512 |
| CM096790 | CAG-TAG | Gln24Term | c.70C>T | p.Q24* | DM | Diabetes, MODY | Osbak (2009) Hum Mutat **30**, 1512 Xiong (2015) *Science* **347**: 1254806 [Additional report] |

PM5: yes

# PP1 criterion (Supporting Pathogenicity )

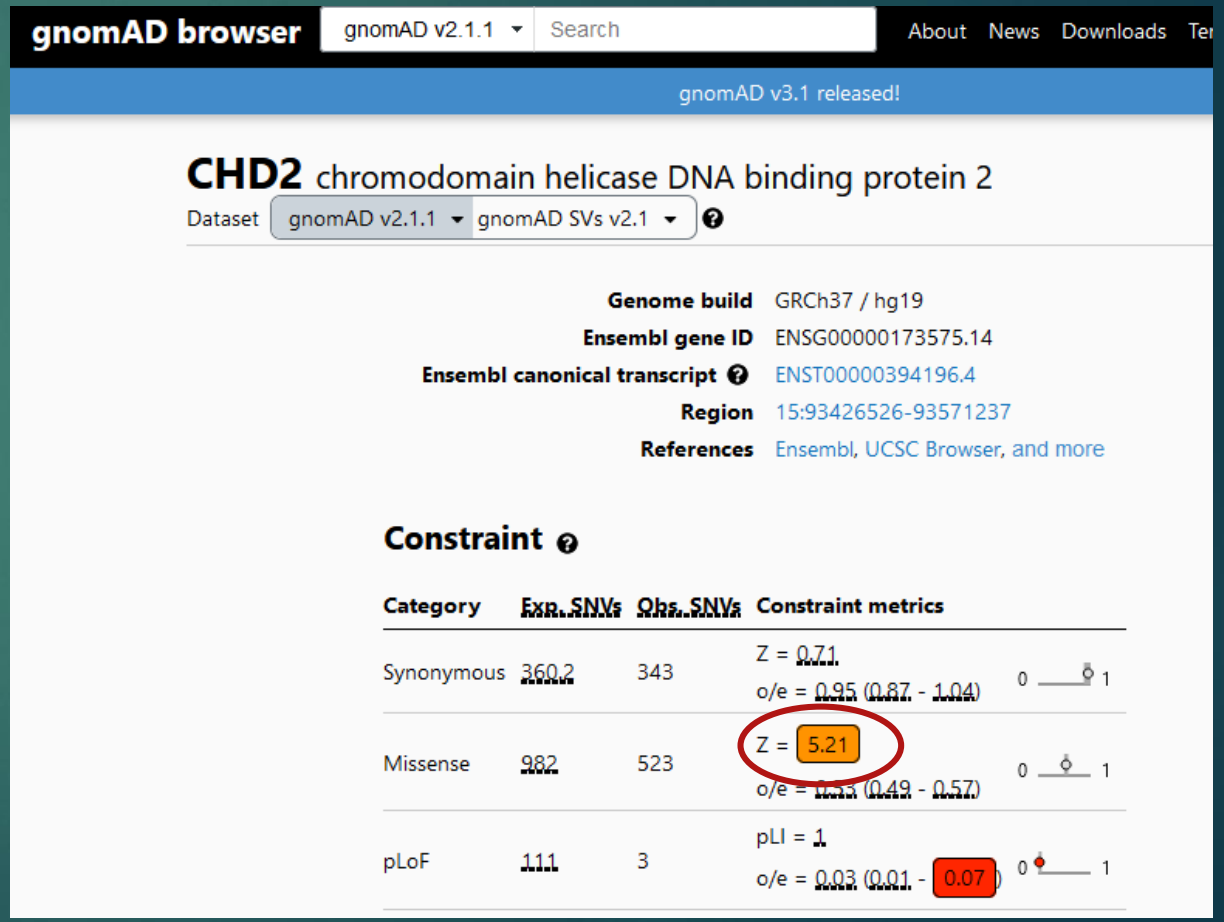
- Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
- Needs sequencing of more than thrre members of the family.
- PP1 = yes if the variant is carried only by ill family's members

# PP2 criterion (Supporting Pathogenicity )

- Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

Where to check:
GnomAD : Constraint function

If Constraint (Missense) Z ≥ 1,75, PP2= yes



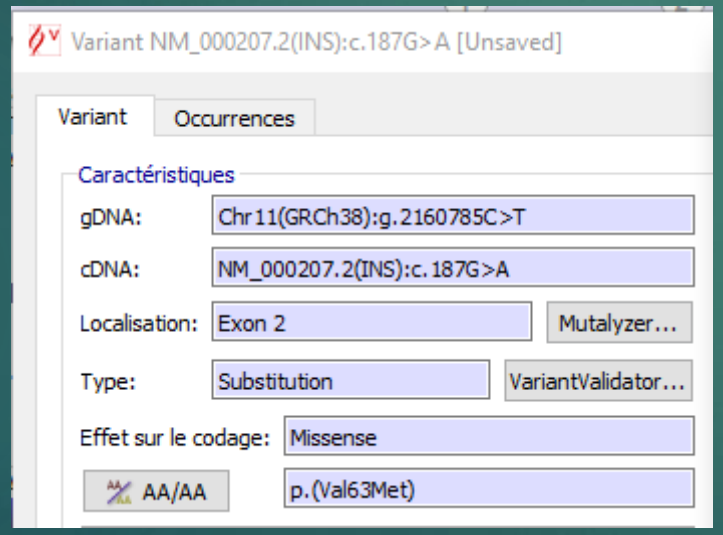
# PP3 criterion (Supporting Pathogenicity )

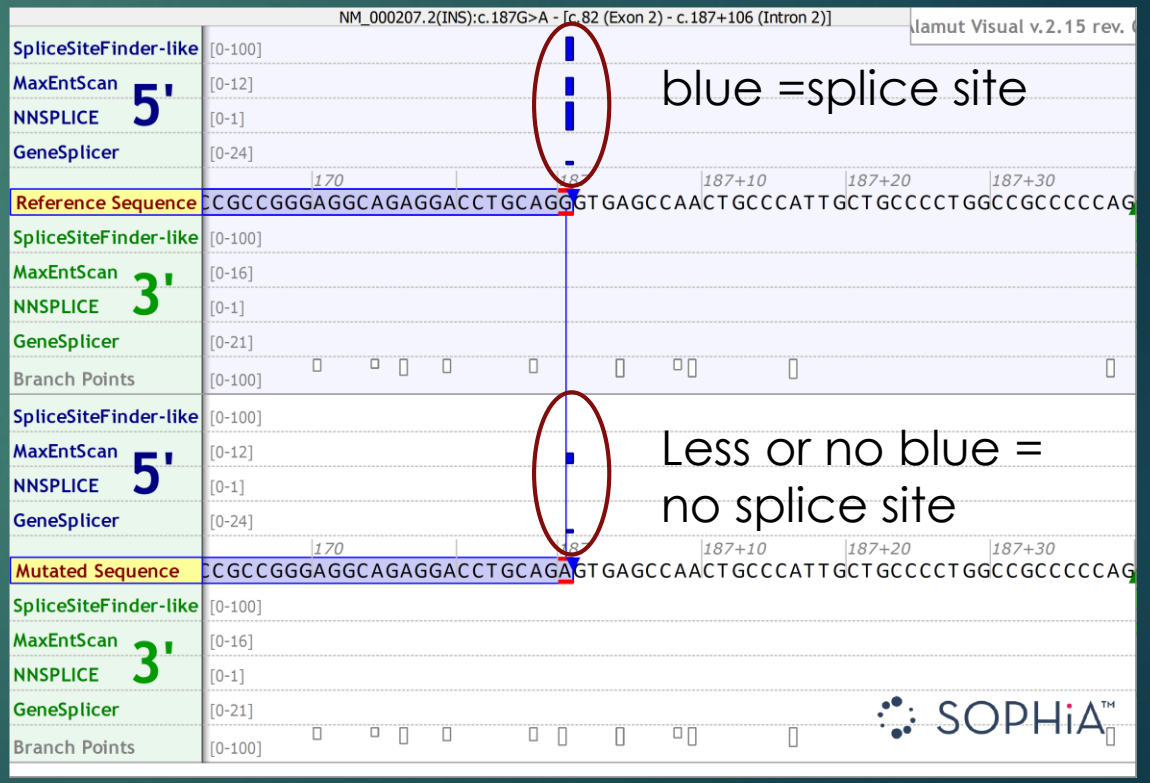
- Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

Splicing predictors via Alamut

Where to check:
- Alamut
- Splicing predictors

Example: splicing effect:

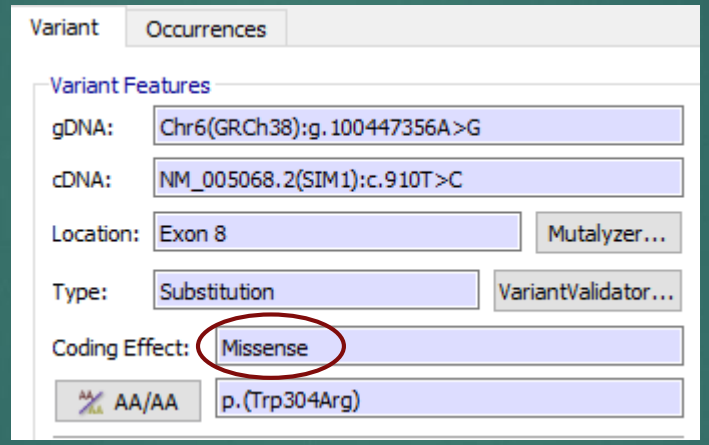


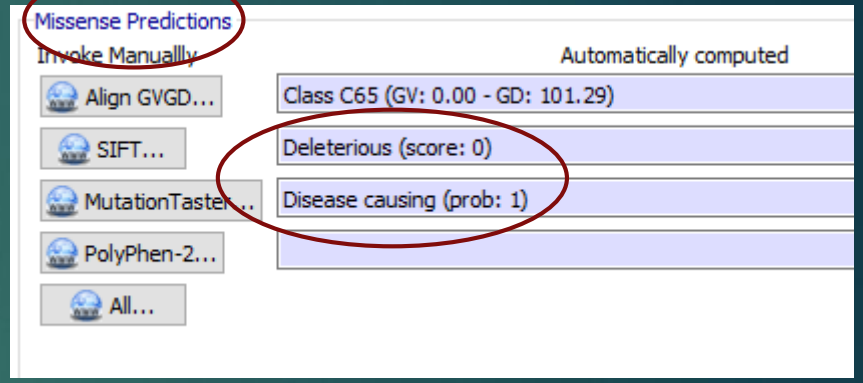



# PP3 criterion (Supporting Pathogenicity )

- Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

Example: misense




Where to check:
- Alamut
- pathogenicity predictors

PP3 = yes if : SIFT: Deleterious
AND
Mutation Taster: Disease Causing

# PP4 criterion (Supporting Pathogenicity )

- Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

  *example: HNf4A, GCK, HNF1A, HNF1B, …* MODY diabetes

# Diagnosis: Rules to classify variants

**Table 5** Rules for combining criteria to classify sequence variants

| | |
|---|---|
| Pathogenic | (i) 1 Very strong (PVS1) *AND*<br>(a) ≥1 Strong (PS1–PS4) *OR*<br>(b) ≥2 Moderate (PM1–PM6) *OR*<br>(c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR*<br>(d) ≥2 Supporting (PP1–PP5)<br>(ii) ≥2 Strong (PS1–PS4) *OR*<br>(iii) 1 Strong (PS1–PS4) *AND*<br>(a)≥3 Moderate (PM1–PM6) *OR*<br>(b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*<br>(c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5) |
| Likely pathogenic | (i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR*<br>(ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR*<br>(iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR*<br>(iv) ≥3 Moderate (PM1–PM6) *OR*<br>(v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR*<br>(vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5) |
| Uncertain significance | (i) Other criteria shown above are not met OR<br>(ii) the criteria for benign and pathogenic are contradictory |

# Diagnosis: Rules to classify variants

**Table 5** Rules for combining criteria to classify sequence variants

| | |
|---|---|
| Pathogenic | (i) 1 Very strong (PVS1) *AND* |
| | (a) ≥1 Strong (PS1–PS4) *OR* |
| | (b) ≥2 Moderate (PM1–PM6) *OR* |
| | (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* |
| | (d) ≥2 Supporting (PP1–PP5) |
| | (ii) ≥2 Strong (PS1–PS4) *OR* |
| | (iii) 1 Strong (PS1–PS4) *AND* |
| | (a)≥3 Moderate (PM1–PM6) *OR* |
| | (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* |
| | (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5) |

Example: selected criteria are:

- PVS1, PM1, PP2 : the variant is pathogenic
- PS1, PS3, PM2: the variant is pathogenic
- PVS1: the variant is VUS (Variant Uncertain Significance)

# Diagnosis: Rules to classify variants

| | |
|---|---|
| Likely pathogenic | (i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR*<br>(ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR*<br>(iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR*<br>(iv) ≥3 Moderate (PM1–PM6) *OR*<br>(v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR*<br>(vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5) |
| Uncertain significance | (i) Other criteria shown above are not met OR<br>(ii) the criteria for benign and pathogenic are contradictory |

Example: selected criteria are:

- PVS1, PM1: the variant is likely pathogenic
- PS1, PM2, PM5: the variant is likely pathogenic
- PM2, PP2, PP3, PP4: the variant is VUS (Variant Uncertain Significance)
- PVS1 only: variant VUS

# Example 1: NM_000162.5(GCK):c.767del // p.Glu256Glyfs*38

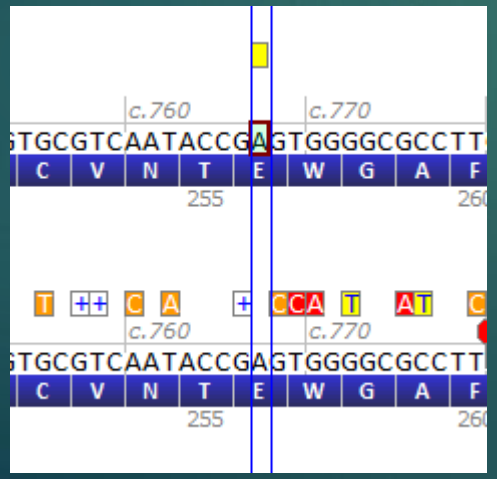
Patient with Type 2 Diabetes
Variant to classify: NM_000162.5(GCK): c.767del // p.Glu256Glyfs*38, ht
Variant not found in controles (GnomAD)
GCK (known gene for diabetes), autosomal dominant

PVS1= yes PM4= no
PM2= yes
PM3 = no

HGMD

| | | | | | | |
|---|---|---|---|---|---|---|
| CD097032 | GGTGGAG^246GGGgACGAGGGCCG | c.739delG | p.(Asp247Thrfs*47) | DM | Diabetes, MODY | Osbak (2009) Hum Mutat 30, 1512 |
| CD178808 | CGAGTGG^258GGCgccTTCGGGGACT | c.775_777delGCC | p.(Ala259del) | DM | Diabetes, MODY | Delvecchio (2017) J Clin Endocrinol Metab 102, 1826 |

ClinVar via Alamut

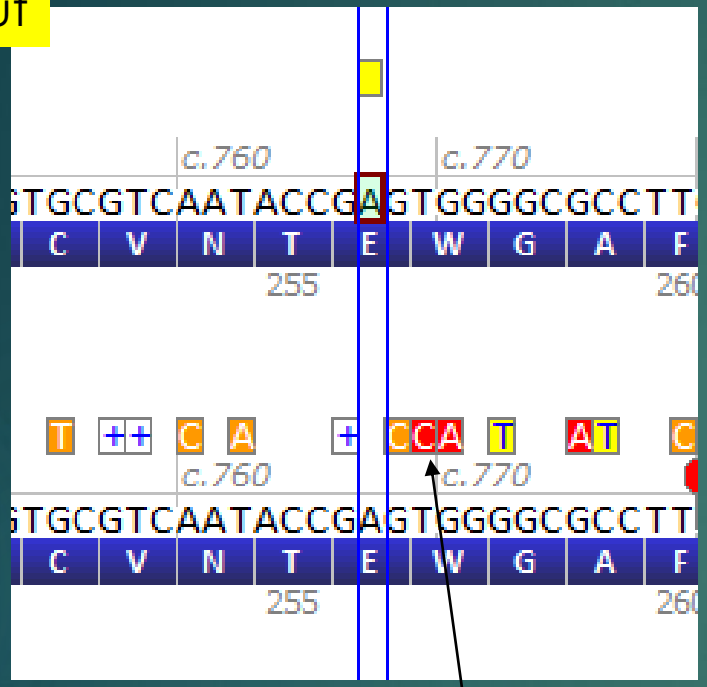



PS1= no
PS3 = no

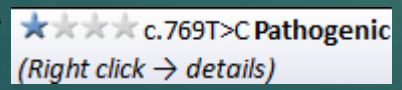
Phenotype patient is highly specific for a monogenic disease PP4= yes

# Example 1 : NM_000162.5(GCK):c.767del // p.Glu256Glyfs*38

Alamut




No missense variant,
PM5 = 0, PP2 = 0




c.769T>C, p.(Trp257Arg)

PM1= yes

# Example 1: conclusion

## NM_000162.5(GCK):c.767del // p.Glu256Glyfs*38

- Criteria:

PVS1

PM1
PM2

PP4

| | | |
|---|---|---|
| Pathogenic | (i) | 1 Very strong (PVS1) *AND* |
| | (a) | ≥1 Strong (PS1–PS4) *OR* |
| | (b) | ≥2 Moderate (PM1–PM6) *OR* |
| | (c) | 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* |
| | (d) | ≥2 Supporting (PP1–PP5) |

This variant is pathogenic for the disease

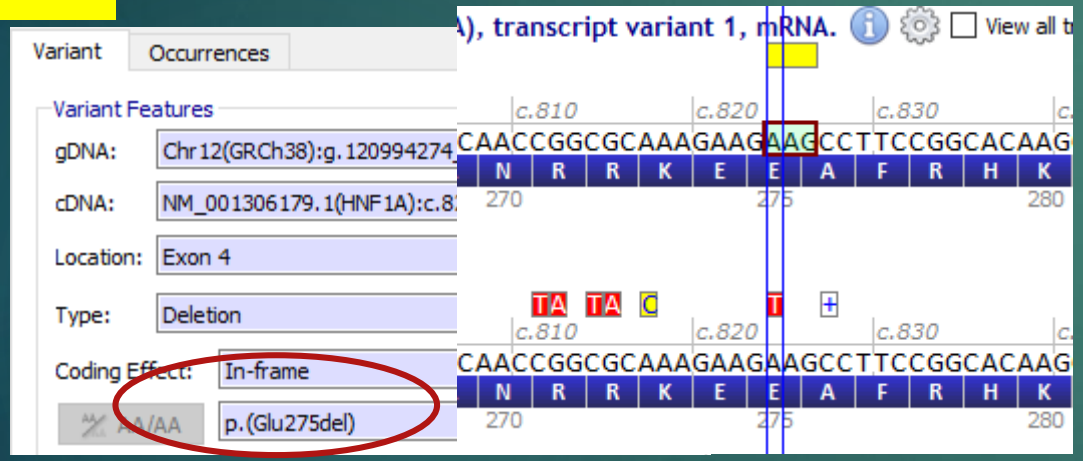
# Example 2

Patient with MODY diabetes (diabetes of the young)
HNF1A (NM_001306179.1):c.824_826del // p.(Glu275del)

Variant found in controles (GnomAD)
HNF1A (known gene for diabetes), autosomal dominant

PM2= no
PM3= no

Alamut




no fs, no stop, no splice, PVS1= NO

In frame, PM4= yes

# Example 2

HNF1A (NM_001306179.1):c.824_826del //p.(Glu275del)

HGMD for indel

| Small deletions | Small insertions | Small indels | Gross deletions | Gross insertions | Complex |
|---|---|---|---|---|---|
| 80 mutations | 51 mutations | 15 mutations | 12 mutations | 2 mutations | 1 mutation |

| HGVS (nucleotide) | HGVS (protein) | Variant class | Reported phenotype | Reference |
|---|---|---|---|---|
| c.815_816delGC | p.(Arg272Glnfs*44) | DM | Diabetes, MODY3 | Lindner (1999) Diabetologia **42**, 121 |
| c.824_826delAAG | p.(Glu275del) | DM | Diabetes, MODY3 | Tatsi (2013) Pediatr Diabetes **14**, 526<br>Flannick (2013) *Nat Genet* **45**: 1380 [Additional report]<br>Najmi (2017) *Diabetes* **66**: 335 [Functional characterisation] |
| c.823_826delGAAG | p.(Glu275Profs*66) | DM | Diabetes, MODY | McKinney (2004) Clin Invest Med **27**, 135 |

PS1 = yes

PS3 = yes

HGMD for missense

| | | | | | | |
|---|---|---|---|---|---|---|
| CGC-AGC | Arg272Ser | c.814C>A | p.R272S | DM | Diabetes, MODY3 | Bellanne-Chantelot (2008) Diabetes **57**, 503 |
| AAA-AAC | Lys273Asn | c.819A>C | p.K273N | DM | Diabetes, MODY | Ellard (2006) Hum Mutat **27**, 854 |
| GAA-GCA | Glu275Ala | c.824A>C | p.E275A | DM? | Diabetes | Bansal (2017) BMC Med **15**, 213 |
| GCC-GAC | Ala276Asp | c.827C>A | p.A276D | DM | Diabetes, MODY | Bjorkhaug (2003) J Clin Endocrinol Metab **88**, 920 |

PM1 = yes in HGMD; presence of missense variant before or after the variant

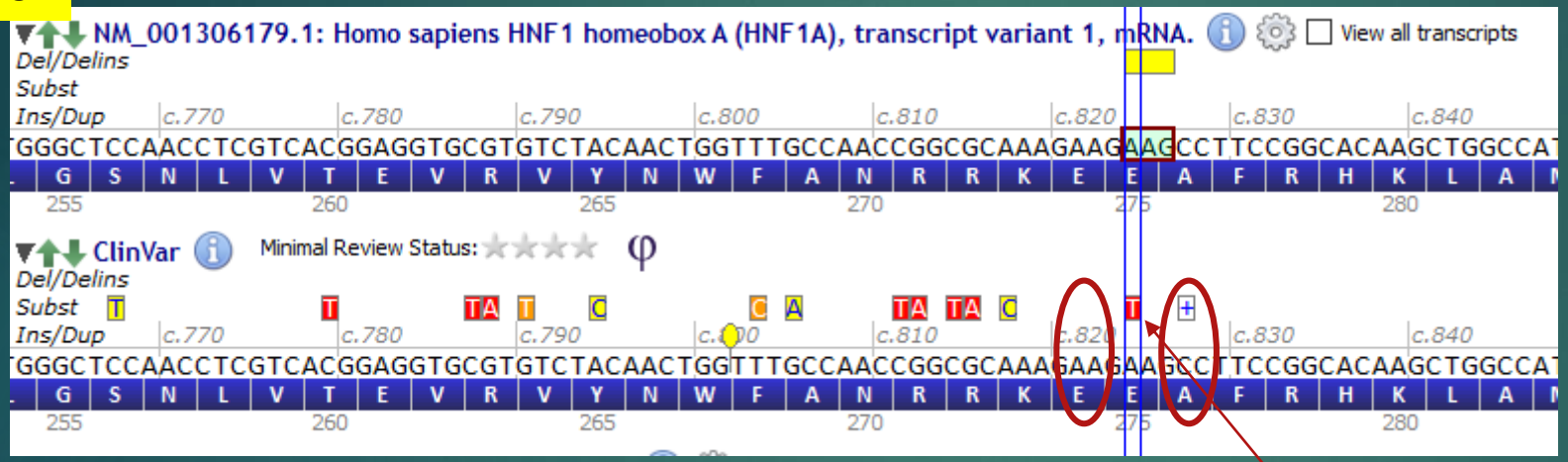
Phenotype patient is highly specific for a monogenic disease

PP4= yes

# Example 2

Patient with MODY diabetes (diabetes of the young)
HNF1A (NM_001306179.1):c.824_826del // p.(Glu275del)

Alamut




- PM1 = NO in clinvar
- PM1 = yes in HGMD
- So PM1 =yes

PM4= yes, no repeat regions

no splice, PVS1= NO

PM5 = no for indel

Phenotype patient is highly specific for a monogenic disease

PP4= yes

# Example 2: conclusion

HNF1A (NM_001306179.1):c.824_826del //p.(Glu275del)

- Criteria:

PS1
PS3

PM1
PM4

PP4

**Table 5** Rules for combining criteria to classify sequence variants

| | |
|---|---|
| Pathogenic | (i) 1 Very strong (PVS1) *AND* |
| | (a) ≥1 Strong (PS1–PS4) *OR* |
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| | (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* |
| | (d) ≥2 Supporting (PP1–PP5) |
| | (ii) ≥2 Strong (PS1–PS4) *OR* |
| | (iii) 1 Strong (PS1–PS4) *AND* |
| | (a) ≥3 Moderate (PM1–PM6) *OR* |
| | (b) 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* |
| | (c) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5) |

This variant is pathogenic for the disease