

Variant classification according to the ACMG Guidelines

The results from the variant
classification in different
Norwegian laboratories.

Mari Ann Kulseth
AMG - OUS

5 variants identified in 3 patients (UK-NEQAS)
were sent out in September
Classification of variants using the ACMG guidelines
16 labs (Genetikportalen)



9 labs responded



5 labs: ACMG-guidelines on daily basis
2 labs: Use the ACMG-guidelines occasionally
1 lab: Does not use the ACMG-guidelines
1 lab: ?



Variant classification

Class 5: pathogenic

Class 4: likely pathogenic

Class 3: VUS – variant of uncertain significance

Class 2: likely benign

Class 1: benign

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

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Disclaimer: These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Standards and Guidelines. They also are advised to take notice of the date any particular guideline was adopted and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

The American College of Medical Genetics and Genomics (ACMG) previously developed guidance for the interpretation of sequence variants.¹ In the past decade, sequencing technology has evolved rapidly with the advent of high-throughput next-generation sequencing. By adopting and leveraging next-generation sequencing, clinical laboratories are now performing an ever-increasing catalogue of genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. By virtue of increased complexity, this shift in genetic testing has been accompanied by new challenges in sequence interpretation. In this context the ACMG convened a workgroup in 2013 comprising representatives from the ACMG, the Association for Molecular Pathology (AMP), and the College of American Pathologists to revisit and revise the standards and guidelines for the interpretation of sequence variants. The group consisted of clinical laboratory directors and clinicians. This report represents expert opinion of the workgroup with input from ACMG, AMP, and College of American Pathologists stakeholders. These recommendations primarily apply to the breadth of genetic tests used in clinical laboratories, including genotyping, single genes, panels,

exomes, and genomes. This report recommends the use of specific standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified in genes that cause Mendelian disorders. Moreover, this recommendation describes a process for classifying variants into these five categories based on criteria using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data). Because of the increased complexity of analysis and interpretation of clinical genetic testing described in this report, the ACMG strongly recommends that clinical molecular genetic testing should be performed in a Clinical Laboratory Improvement Amendments–approved laboratory, with results interpreted by a board-certified clinical molecular geneticist or molecular genetic pathologist or the equivalent.

Genet Med advance online publication 5 March 2015

Key Words: ACMG laboratory guideline; clinical genetic testing; interpretation; reporting; sequence variant terminology; variant reporting

ACMG-Guidelines

16 pathogenic criteria

12 benign criteria

Very strong

PVS1 – LOF

Strong

PS1 – same aa change known
PS2 – de novo
PS3 – functional test
PS4 – affected individuals

Moderate

PM1 – mutation hotspot
PM2 – absent from controls
PM4 – change in protein length
PM5 – other aa change in same codon
PM6 – de novo

Supporting

PP1 – cosegregation
PP2 – rare benign missense
PP3 – in silico evidence
PP4 – phenotype match
PP5 – source without evidence

Stand alone

BA1 – MAF > 5%

Strong





BS1 – MAF > prevalence
BS2 – in healthy individuals
BS3 – functional test
BS4 – lack of cosegregation




Supporting

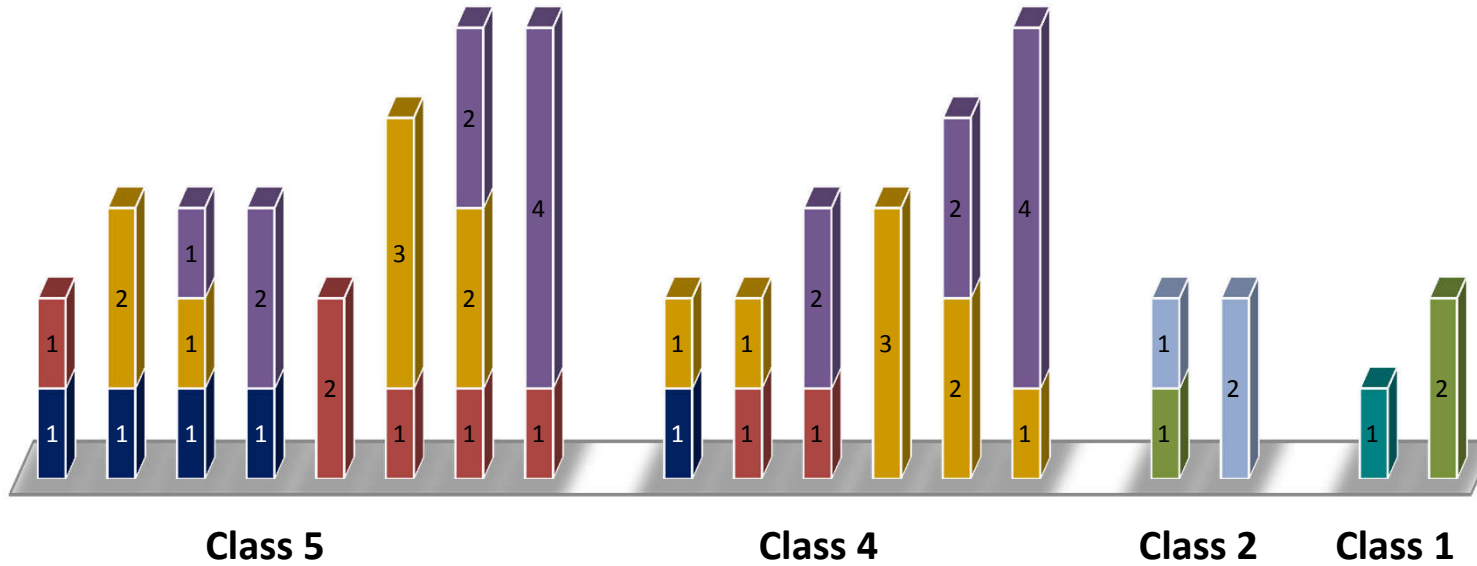
BP1 – missense in LOF gene
BP2 – trans (AD) /cis (AR)
BP3 – repetitive region
BP4 – in silico evidence
BP5 – other known case of disease
BP6 – source without evidence
BP7 – synonymous without splicing effect

16 pathogenic criteria

12 benign criteria

-  Very strong
-  Strong
-  Moderate
-  Supporting

-  Stand alone
-  Strong
-  Supporting



The strength of each criteria might vary, depending on the evidence itself and/or the context it is going to be used.



Phenotype: Severe infantile - full or reduced penetrance - variable expressivity -
late onset - phenocopies


Inheritance: Dominant - *de novo* - Recessive - X-linked

Control population: Absent - a few - MAF < prevalence

Reported affected individuals: two - three - five - ten -

Patient 1

Elizabeth Braddock (dob 27/11/1958) is presenting with an 8 year history of cognitive decline characterized by problems with short term memory and navigation. Her mother was also clinically diagnosed with early onset dementia. Testing for Alzheimer disease has been requested by a consultant neurologist and the results obtained are as follows:

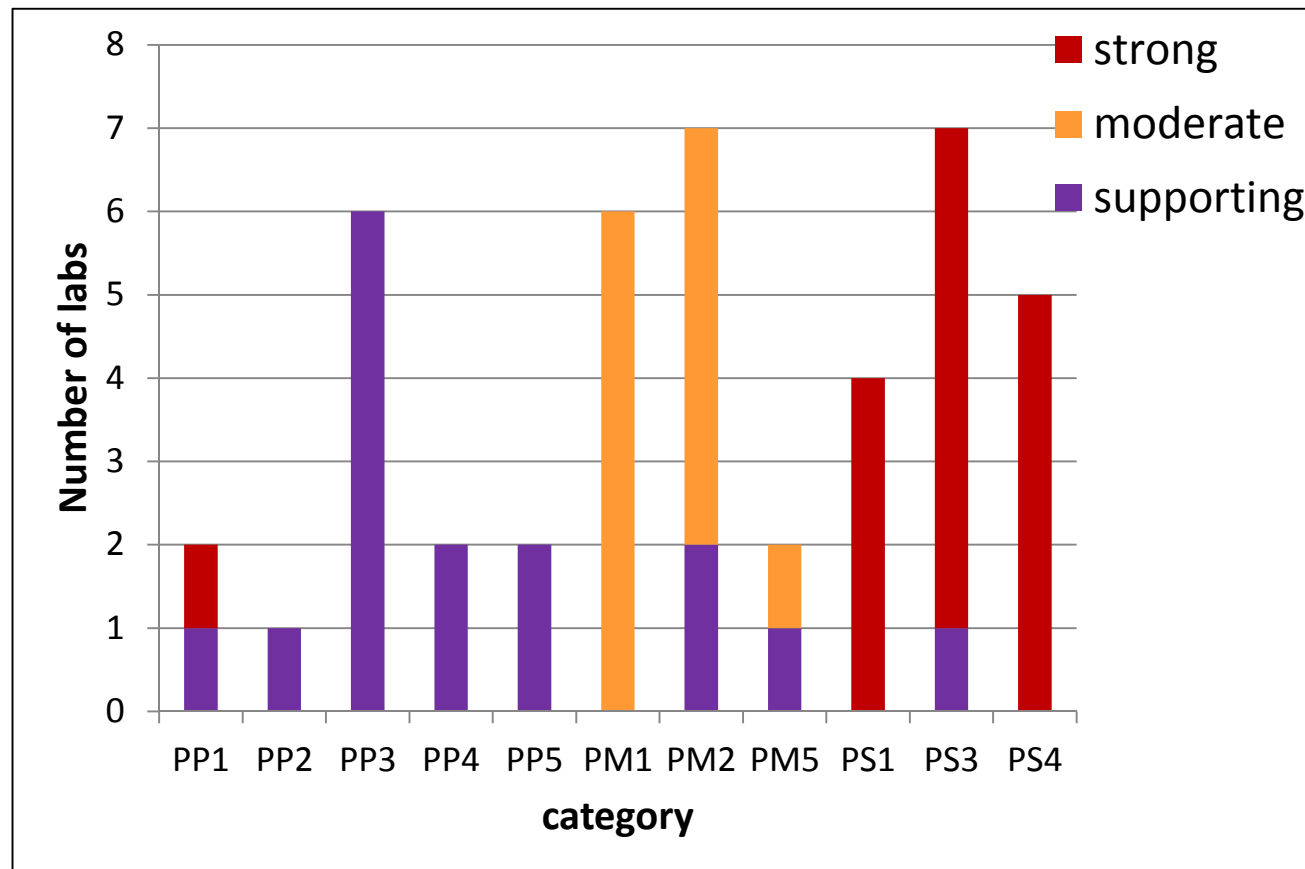


APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygous
PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygous

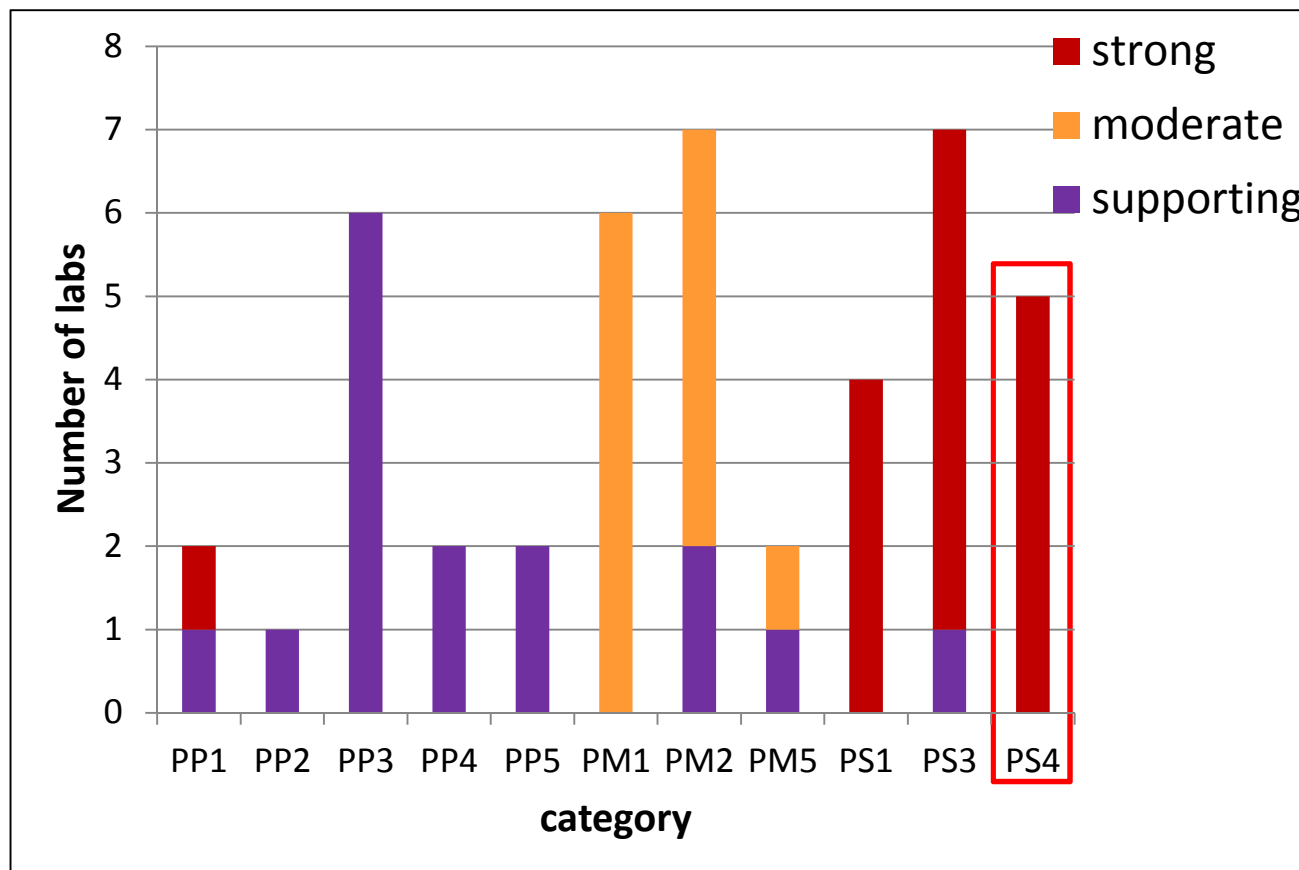
APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

APP – autosomal dominant inherited Alzheimer disease 1 (OMIM#104300)

All 9 labs suggested class 5 !!!

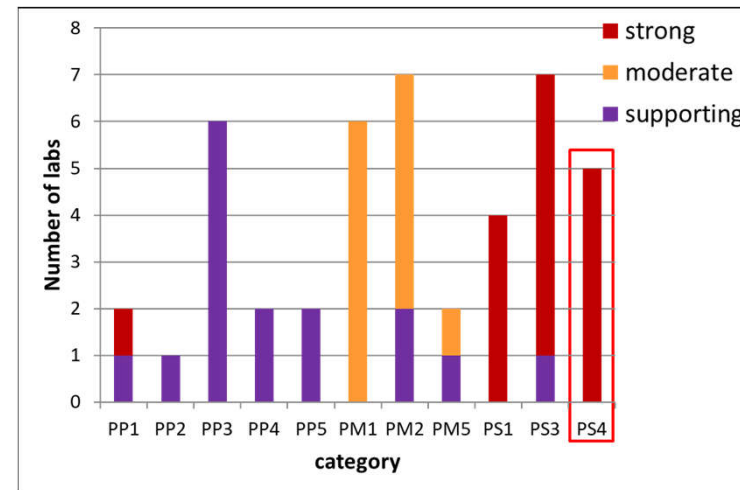


APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot



PS4 - The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

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.



Labs 3, 4, 5, 8 and 9: Reported in a significant number of patients and not in gnomAD

| Lab | Category | Evidence |
|-----|----------|---|
| 1 | PS1 | Aminosyreendringen publisert som patogen |
| 2 | PS1 | Same amino acid change |
| 6 | PM5 | Samme codon: Val717Leu, Val717Phe, Val717Gly |
| 7 | PS1 | Samme aminosyre-endring er tidligere etablert som patogen |

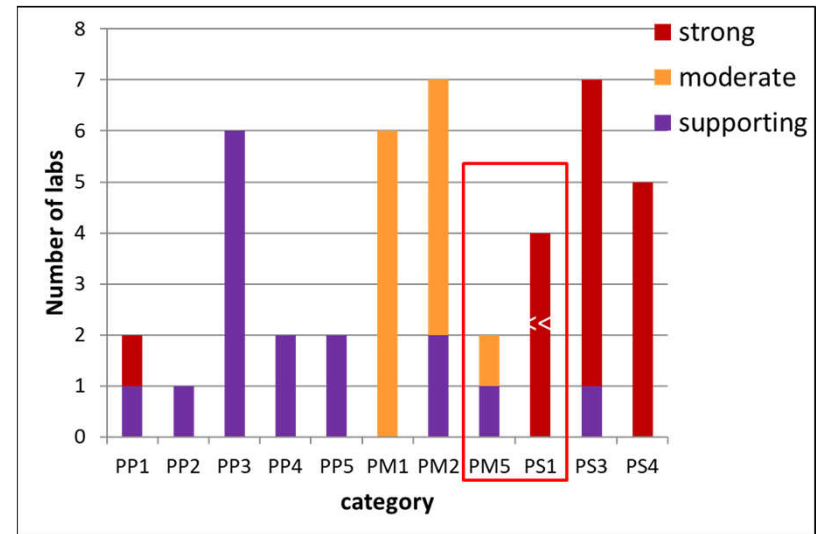
APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

PS1 - Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val > Leu caused by G>C or G>T in same codon.

PM5 - 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



HGMD/ClinVar

| | | |
|---------------------|--------------------|---------------------|
| GTC > GGC | p.Val717Gly | c.2150T>G |
| GTC > ATC | p.Val717Ile | c.2149G>A |
| GTC > CTC | p.Val717Leu | c.2149G>C |
| GTC > TTC | p.Val717Phe | c.2149G>T |

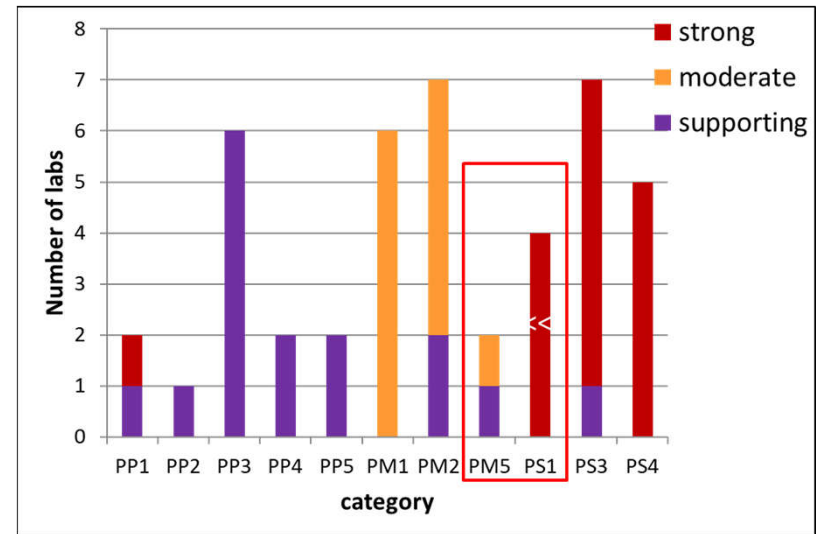
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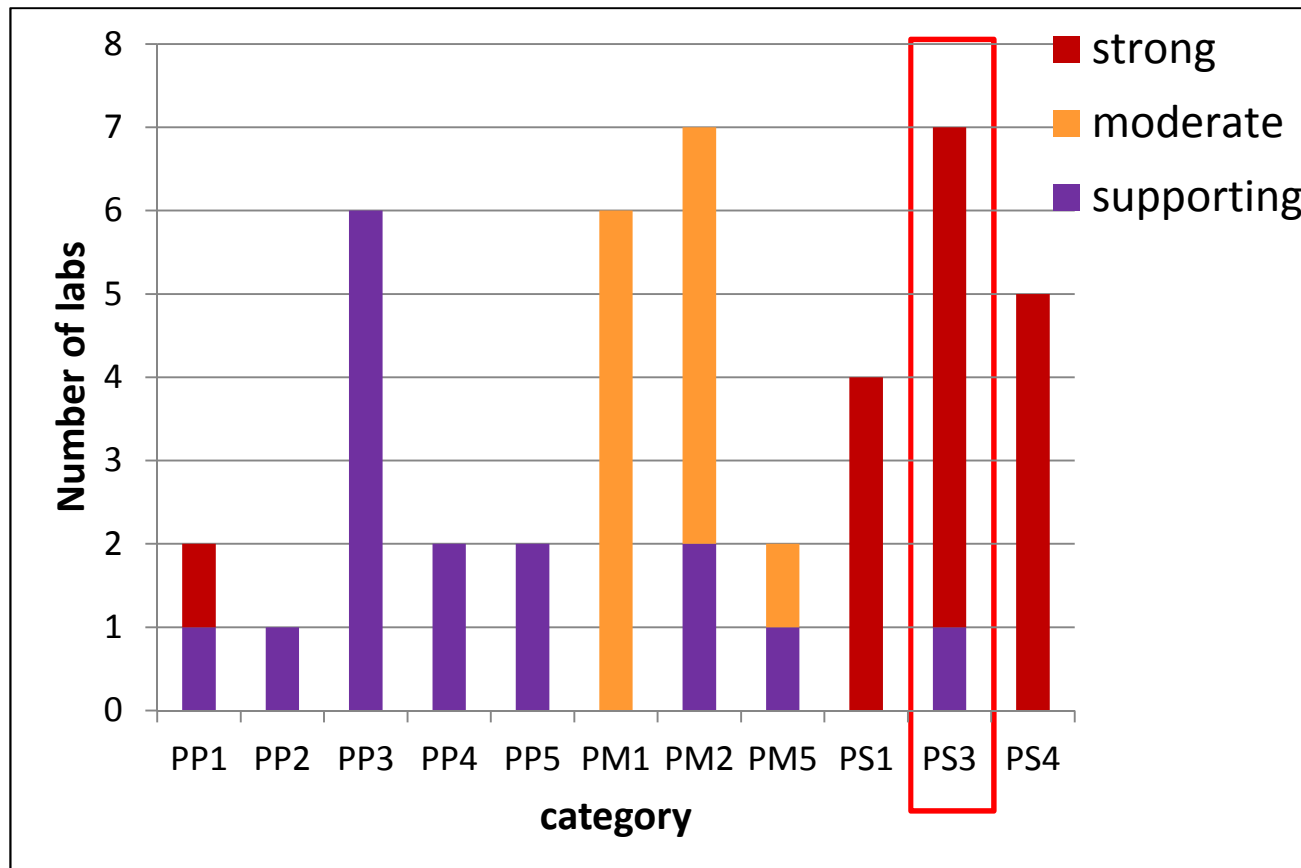
Example: Arg156His is pathogenic; now you observe Arg156Cys



HGMD/ClinVar

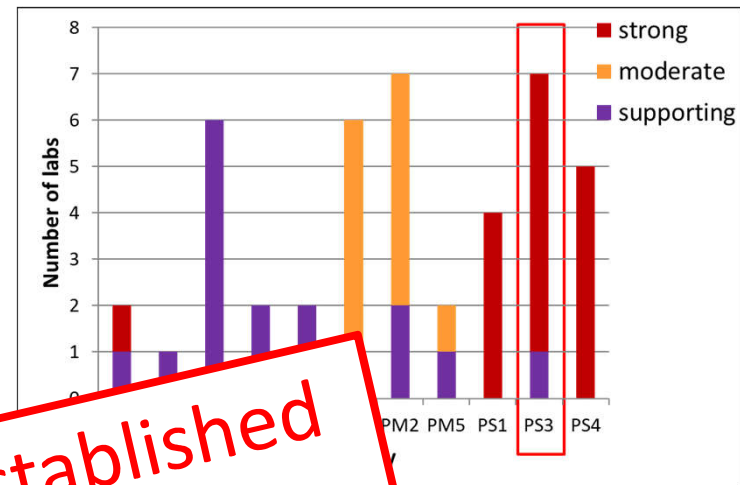
| | | |
|---------------------|--------------------|---------------------|
| GTC > GGC | p.Val717Gly | c.2150T>G |
| GTC > ATC | p.Val717Ile | c.2149G>A |
| GTC > CTC | p.Val717Leu | c.2149G>C |
| GTC > TTC | p.Val717Phe | c.2149G>T |

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot



APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

PS3 - Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product



6 labs used PS3 – strong

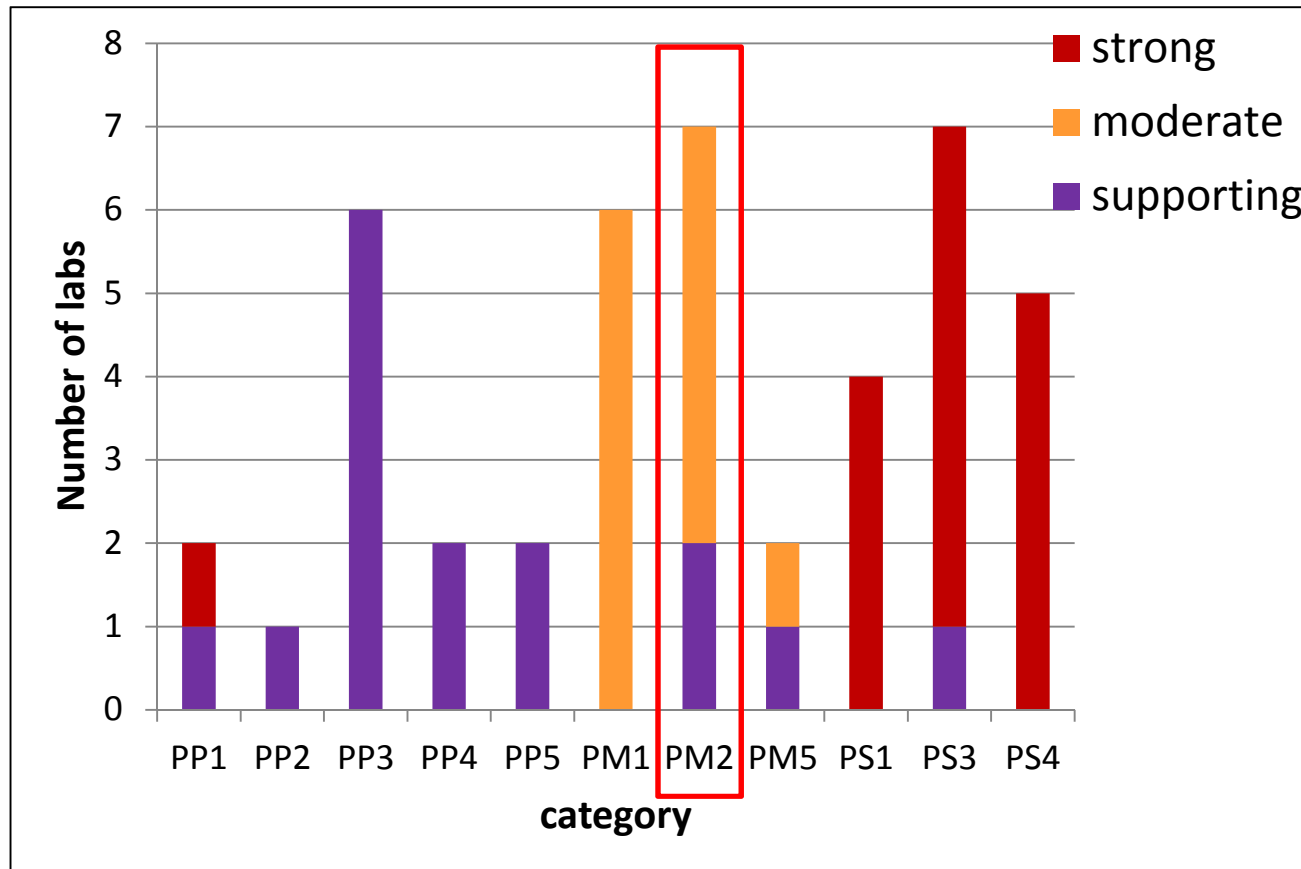
| lab | evidence |
|-----|---|
| 1 | Veletablerte funksjoner |
| 2 | In vitro |
| 4 | In vitro/in vivo funksjoner som viser en ødeleggende effekt på mRNA eller protein |
| 6 | Litteratur: 8 publ. støtter varianten (HGMD Prof) |
| 8 | Funksjonelle studier viser at varianten har en ødeleggende effekt på proteinfunksjonen |
| 9 | Funksjonelle studier indikerer Alzheimer-relevant effekt på proteinet, økt ratio av APP som akkumulerer |

What is a well-established functional study?

1 lab used PS3 – supporting

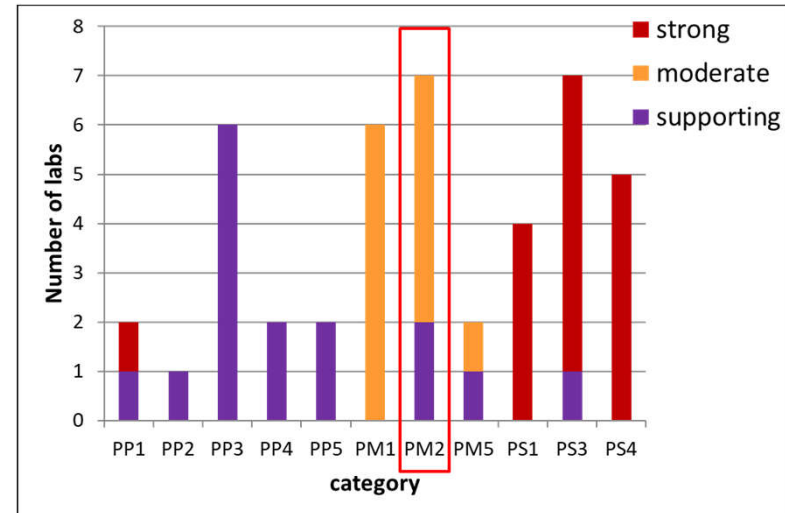
| | |
|---|---|
| 5 | Flere funksjonell studier. Dimitrov (2013) Nat Commun 4: 2246 |
|---|---|

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot



APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

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



Labs 1,2,4,6 and 7 – moderate – absent from gnomAD

Labs 5 and 8 – supporting – absent from gnomAD, but ethnicity unknown

ACMG-Guidelines: “If a variant is absent from (or below the expected carrier frequency if recessive) a large general population or a control cohort (>1,000 individuals) and **the population is race-matched to the patient harboring the identified variant**, then this observation can be considered a moderate piece of evidence for pathogenicity (PM2).

In ExAC (60 706 humans): 7.4 mill high quality variants in the exomes

Approximately 50% were novel singletons

→ **60 novel variants per exome**

(Lek M et al., Nature 2016; 536:285)

Whole-exome sequencing of 176 individuals from island of Vis in Croatia

identified 25 430 novel SNVs not seen in esp, UK10K, 1000 Genome, ExAc or dbSNP.

→ **144 novel variants per exome**

(Jeronic A et al., Eur J Hum Genet 2016; 24: 1479)

If we do exome sequencing, should all these novel variants be weighted as moderate evidence for pathogenicity?

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

PS4 - Reported in a significant number of patients and not in gnomAD



PM2 - Absent from controls (gnomAD)

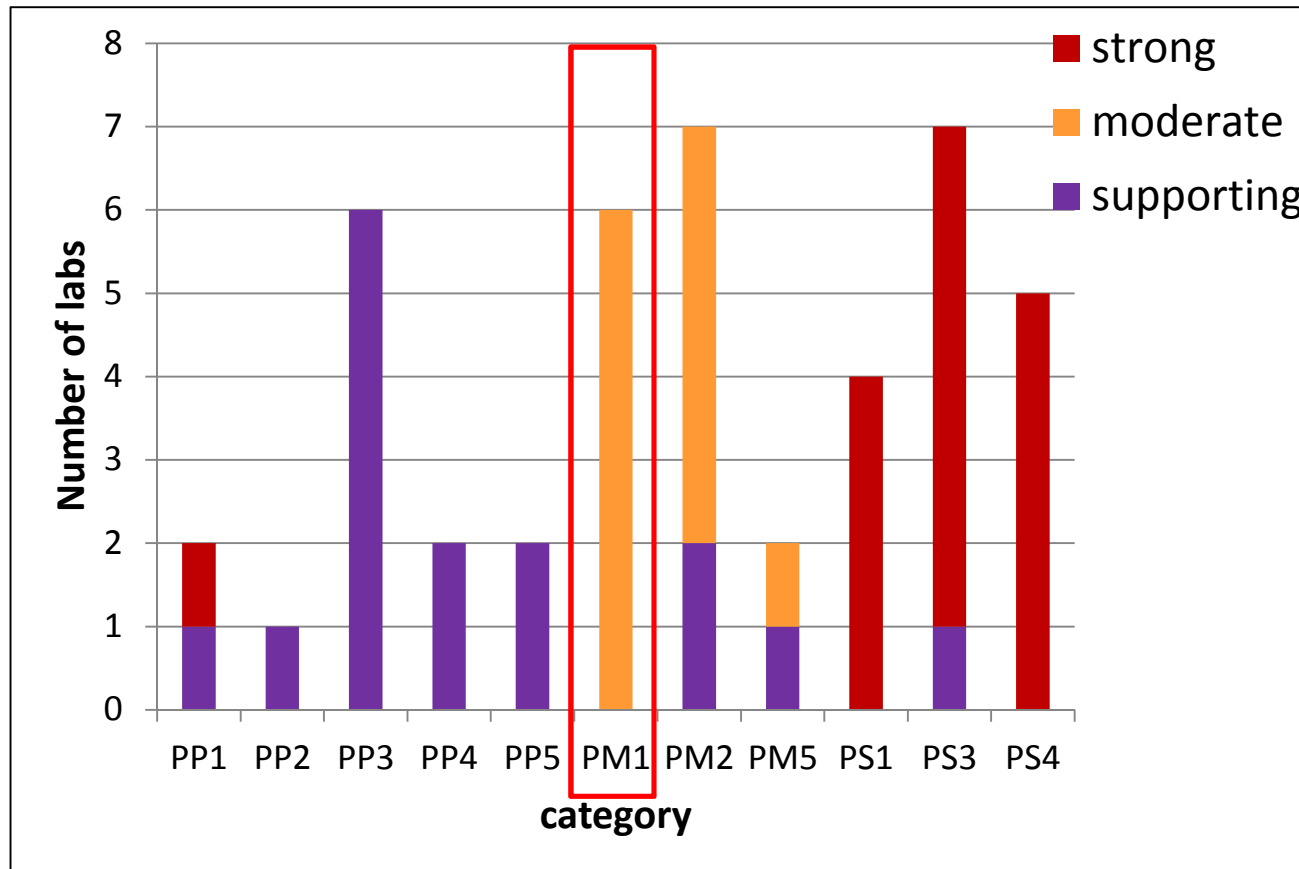
«double counting»?

Labs 5 and 8 – supporting – absent from gnomAD, but ethnicity unknown
Labs 1,2,4,6 and 7 – moderate – absent from gnomAD

Labs 3 and 9 have used PS4

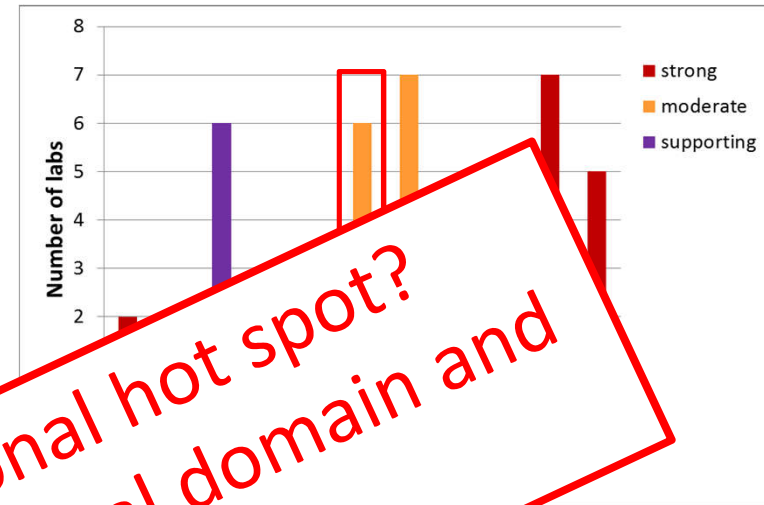
Did not use PM2 due to «double counting».

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot



APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

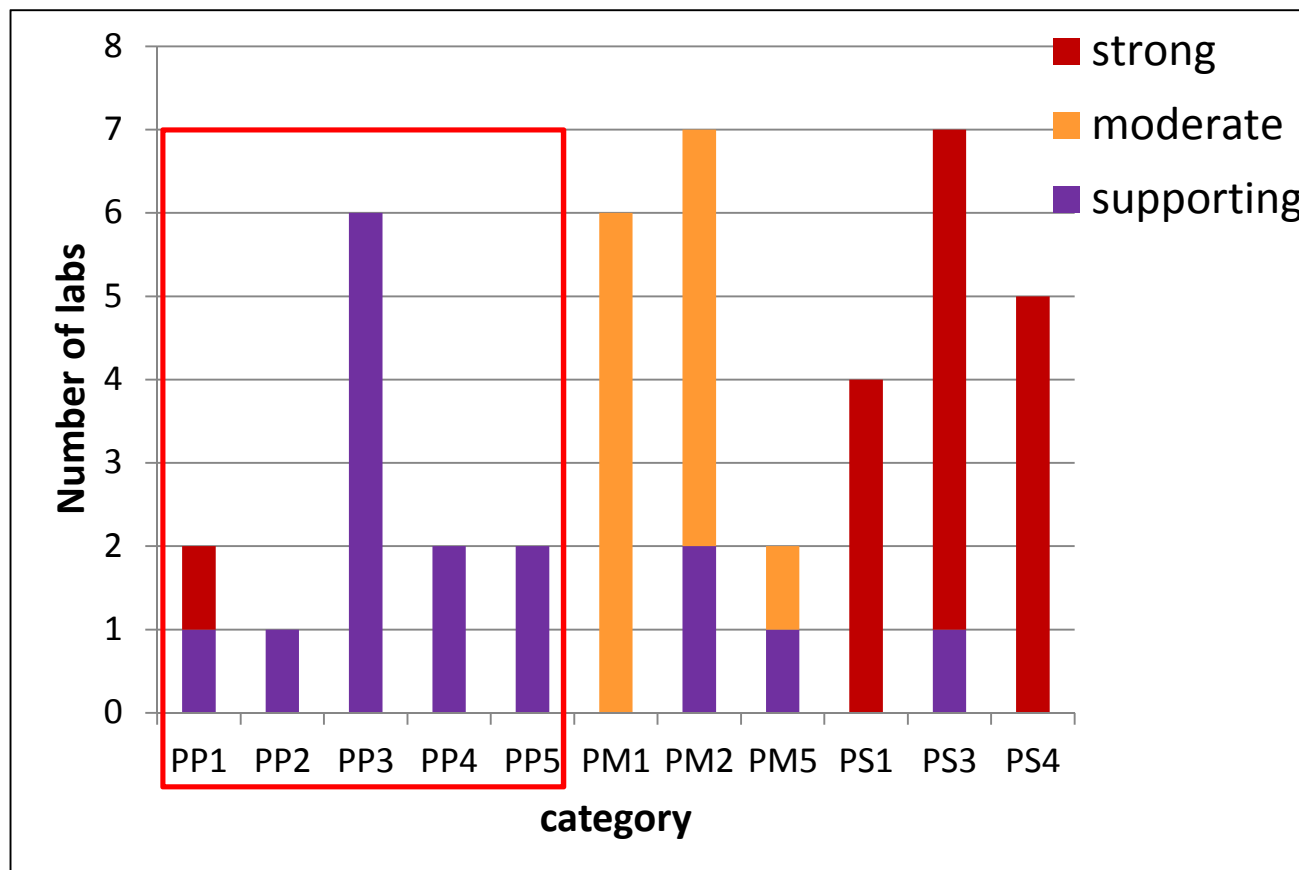
PM1 - Located in a mutational hot spot and/or critical and well-established functional domain without benign variation



| Lab | Evidence |
|-----|---|
| 3 | Mutational Hotspot |
| 4 | Lokalisert i mutasjonsregioner i funksjonelt domene i proteinet (f.eks., aktivt sete i ...) |
| 5 | Lokalisert i mutasjonsregioner i funksjonelt domene i proteinet (f.eks., Gorn ... (APP-TM dimer, viktig aminosyre) PMID: 18234110) |
| 6 | Mutasjon i mutasjonsregioner i funksjonelt domene i proteinet (f.eks., (Biomarkers for Alzheimer's Research & Therapy 2012, 4:9); Ryan & Rossor (1999-112). mutational hotspot region around the γ -secretase cleavage site; APP ... just distal to the C-terminus of the A β domain by the γ -secretase site, was recognized ... present a mutation hotspot. |
| 7 | Mutasjon er lokalisert i hotspot (Oliveira et al, 2010 Hum Mut) |
| 9 | Mutational hotspot. Er i ekson 17. Tilnærmet alle sykdomsgivende varianter i APP er i ekson 16 eller 17, som koder for det proteolytisk kløyvede A-beta peptidet. Flere andre dokumenterte sykdomsgivende missense i samme posisjon (Val717Leu, Val717Phe, Val717Gly), som er omtrent like dramatiske endringer som Val717Ile |

What defines a mutational hot spot?
 What is a critical functional domain and
 how do we know?

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot



PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

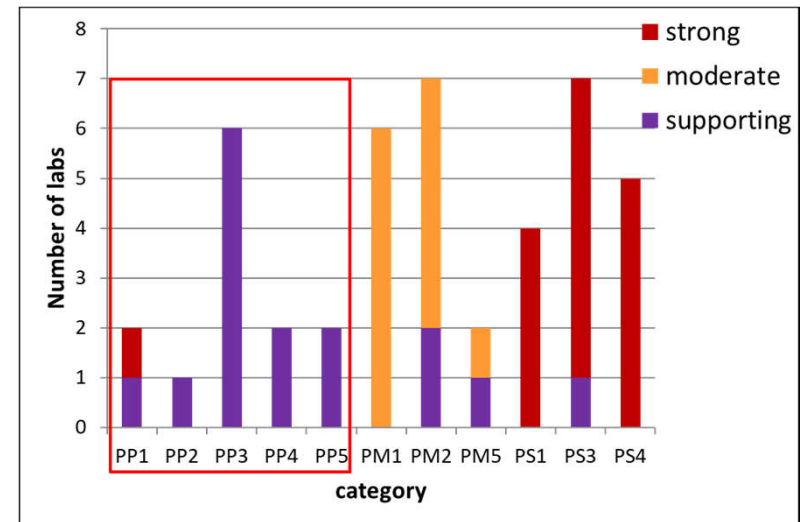
Note: May be used as stronger evidence with increasing segregation data

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

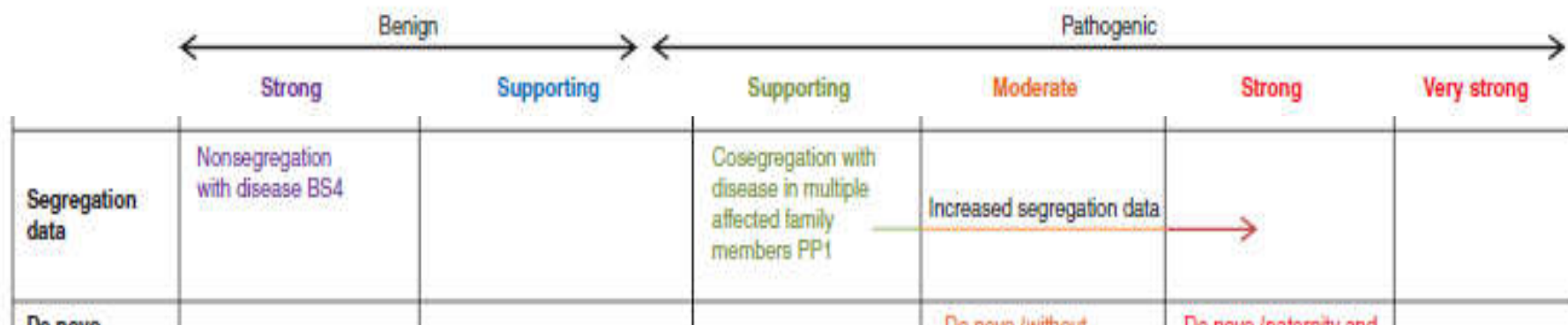
PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation



Lab 9: PP1 – strong –cosegregation with Alzheimer in several large families



Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants

Gail P. Jarvik^{1,*} and Brian L. Browning¹

The American College of Medical Genetics and Genomics (ACMG) and Association of Molecular Pathology (AMP) recently published important new guidelines aiming to improve and standardize the pathogenicity classification of genomic variants. The Clinical Sequencing Exploratory Research (CSER) consortium evaluated the use of these guidelines across nine laboratories. One identified obstacle to consistent usage of the ACMG-AMP guidelines is the lack of a definition of cosegregation as criteria for pathogenicity classification. Cosegregation data differ from many other types of pathogenicity data in being quantitative. However, the ACMG-AMP guidelines do not define quantitative criteria for use of these data. Here, such quantitative criteria, in an easily implementable form, are proposed.

Introduction

The consideration of cosegregation of a genetic variant and disease is important data when evaluating the pathogenicity of a genomic variant. Thus, cosegregation is included as part of the recently published, important American College of Medical Genetics and Genomics (ACMG) and Association of Molecular Pathology (AMP) guidelines aiming to improve and standardize the pathogenicity classification of genomic variants.¹ Such guidance is a crucial step in advancing a consistent implementation of genomic medicine.

The ACMG-AMP pathogenicity classification guidelines offer a set of categories that can each be used to offer vary-

The goal of this work is to propose a set of easily implemented, quantitative guidelines for the consideration of cosegregation of a variant and a disease in the classification of variant pathogenicity. These proposed guidelines support specific ACMG-AMP evidence levels. These guidelines are designed to be implementable by molecular pathologists and clinical geneticists without advanced statistical genetics training.

Material and Methods

Although the Thompson-Bayrak-Toydemir BF method can achieve

PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

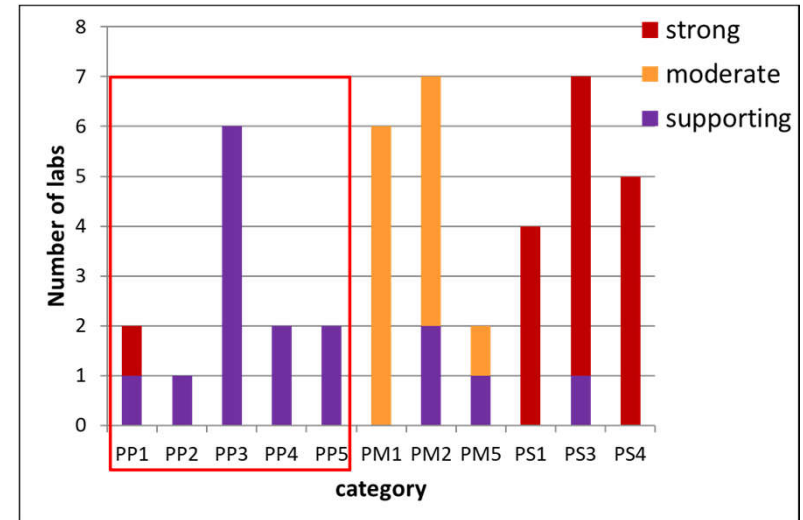
Note: May be used as stronger evidence with increasing segregation data

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

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PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation



Lab 7: missense in a gene with few missense mutations

ExAC:

Expected no. missense 293

Observed no. missense 228

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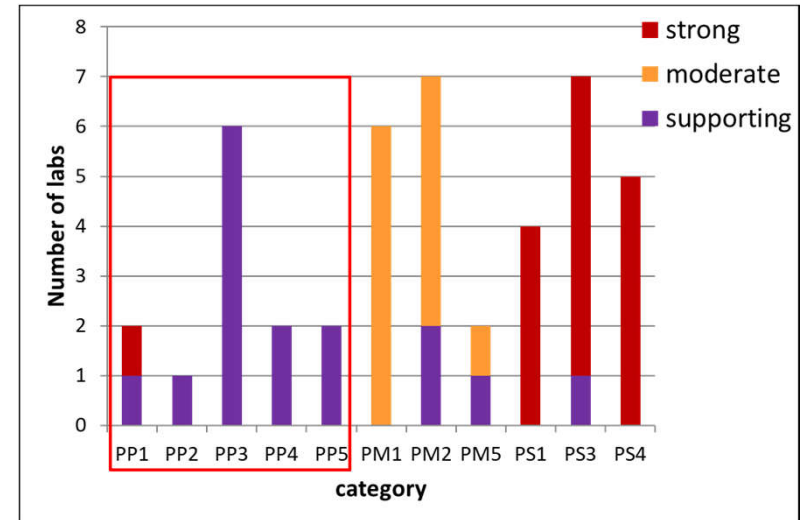
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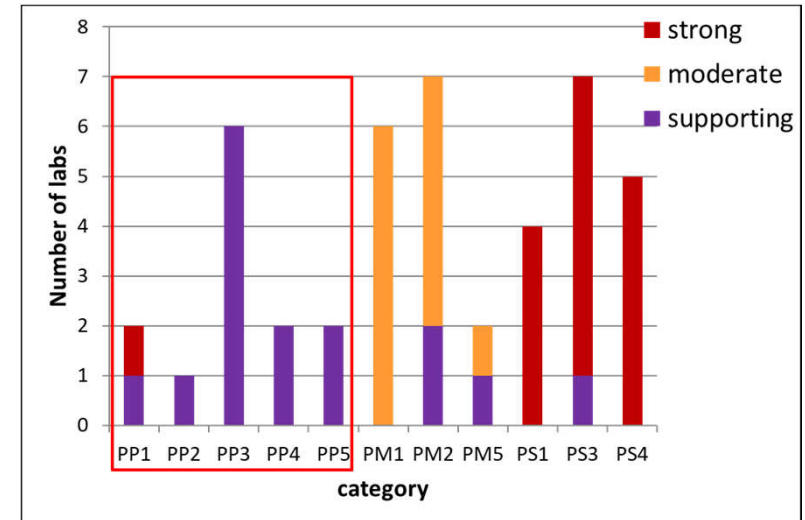
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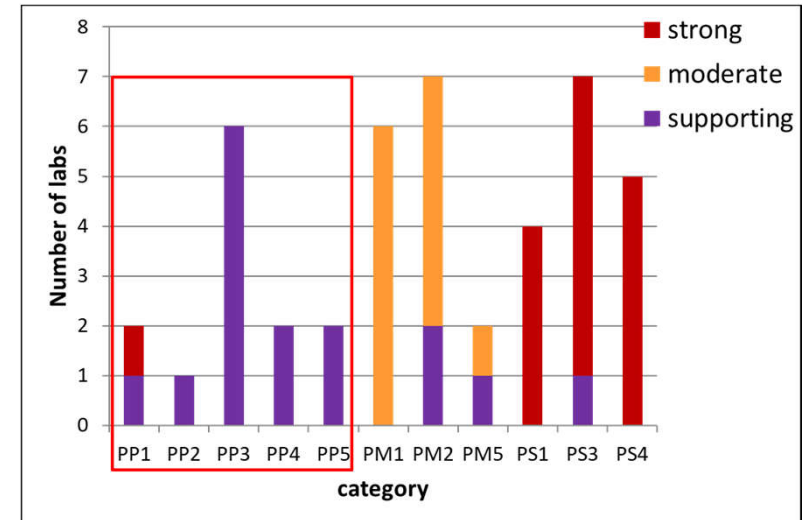
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Lab 3 – pathogenic for Alzheimer in ClinVar

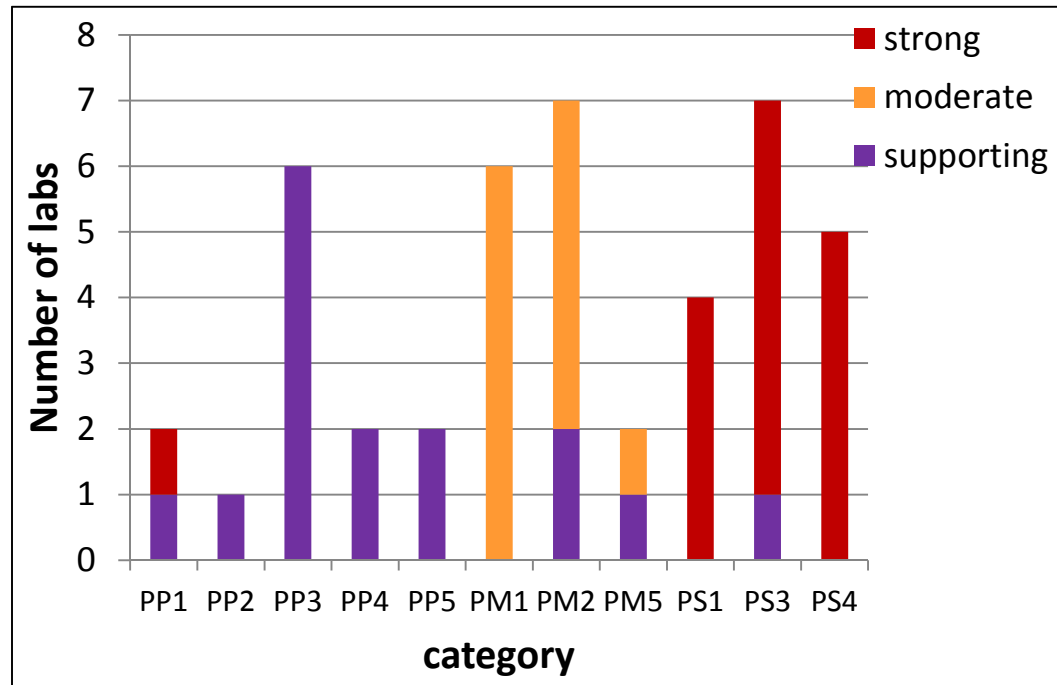
Lab 6 – ClinVar: 3x pathogenic but without information

ClinVar 2 pathogenic with reference to 8 and 6 papers in PubMed

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygot

APP – autosomal dominant inherited Alzheimer disease 1 (OMIM#104300)

All 9 labs suggested class 5!!!



The choice of categories and weighting used for classification, varied a bit more than expected and perhaps was somewhat creative.

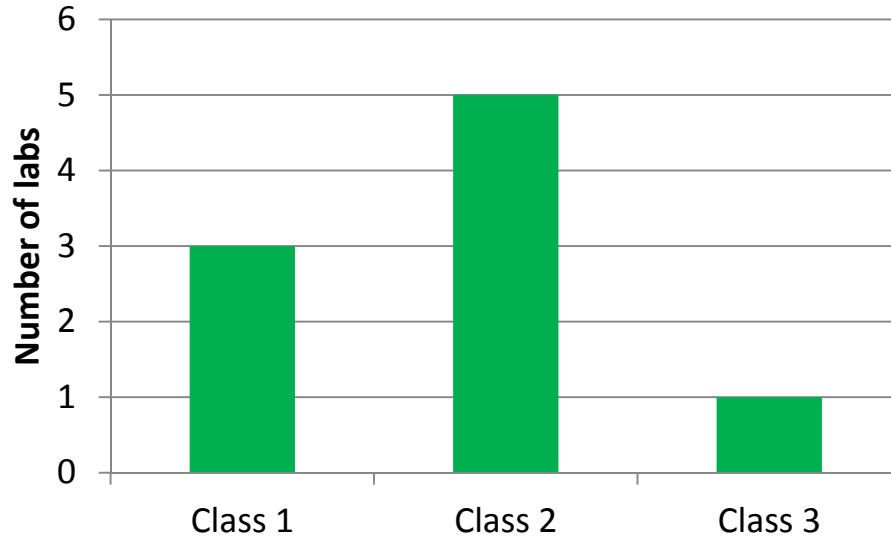
Patient 1

Elizabeth Braddock (dob 27/11/1958) is presenting with an 8 year history of cognitive decline characterized by problems with short term memory and navigation. Her mother was also clinically diagnosed with early onset dementia. Testing for Alzheimer disease has been requested by a consultant neurologist and the results obtained are detailed in the table below.

APP (NM_000484.3) c.2149G>A (p.Val717Ile) heterozygous
PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygous



PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygot



Class 1 – benign

Class 2 – likely benign

Class 3 – variant of uncertain significance

PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygot

Class 1 - benign

| Lab | Category | strength | evidence |
|-----|----------|-----------------|---|
| 5 | BS1* | Frittstående | Høy frekvens (0.03356 i European (Finnish) gnomAD, >0,5% for AD arv gir frittstående BS1 ved vår lab) |
| 7 | BA1 | Sterk nok alene | Allelfrekvens mer enn 0,5% i ExAc (1,86% for rs17125721) |
| | BS1 | Sterk | Allelfrekvensen høyere enn forventet for sykdommen |
| | BS2 | Sterk | Påvist i friske voksne |
| 9 | BS1* | «Stand alone» | Klasse 1 basert på egne kriterier – AD, AF over 0,5% settes til 1 |

* In house criteria BS1 for frequency > 0,5% for variants in AD genes. Used as stand alone criteria

ACMG Guideline:

BA1 (stand alone criteria)– Allele frequency is > 5% in gnomAD

Lab adopted guideline:

BA1 (BS1*) – Allele frequency is > 0,5% (AD) in gnomAD

PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygot

Class 1 - benign

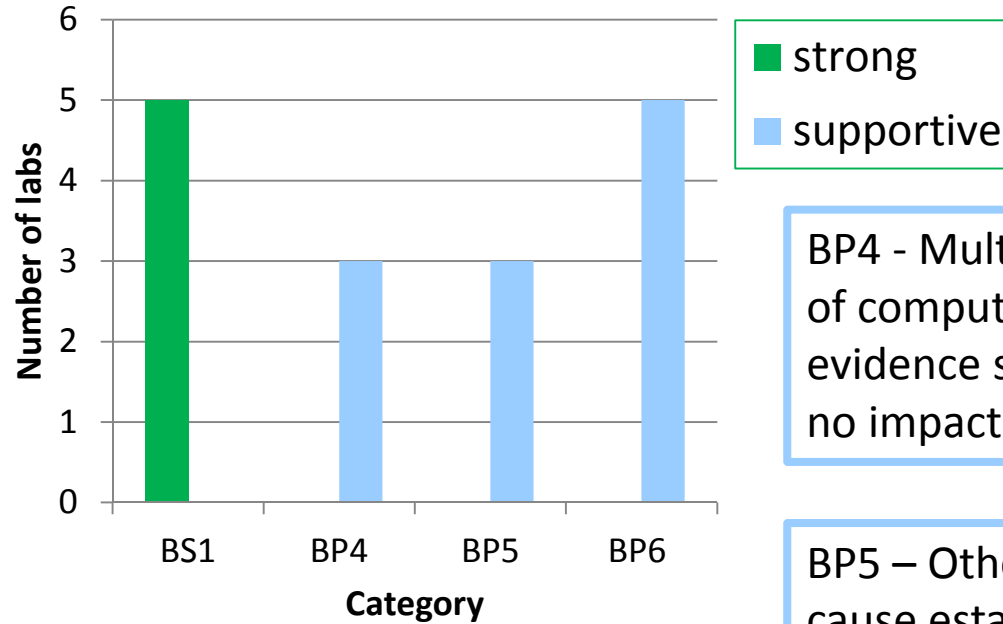
| Lab | Category | strength | evidence |
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* In house criteria BS1 for frequency > 0,5% for variants in AD genes. Used as stand alone criteria

GnomAD :

| Population | Allele Count | Allele Nu. | Nu. of Hom | Allele Frequency |
|-------------------|--------------|------------|------------|------------------|
| European (Fi) | 865 | 25772 | 16 | 0.03356 |
| Ashkenazi Jewish* | 248 | 10146 | 6 | 0.02444 |
| European (Non-Fi) | 2416 | 126264 | 16 | 0.01913 |
| Other | 112 | 6452 | 2 | 0.01736 |
| South Asian | 189 | 30748 | 2 | 0.006147 |
| Latino | 209 | 34406 | 2 | 0.006075 |
| African | 69 | 24006 | 1 | 0.002874 |
| East Asian | 1 | 18850 | 0 | 0.00005305 |
| Total | 4109 | 276644 | 45 | 0.01485 |

PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygot
Class 2 – likely benign



BS1 - MAF > expected for disease

BP4 - Multiple lines of computational evidence suggest no impact

BP5 – Other genetic cause established

BP6 – Reputable source have reported the variant as benign

PSEN1(NM_000021.3) c.953A>G (p.Glu318Gly) heterozygot


Lab 8: Class 3 – uncertain significance

BS1 – strong – MAF > expected for disease

The variant is a risk factor for late-onset Alzheimer disease
(ACMG criteria not suited).

Patient 2

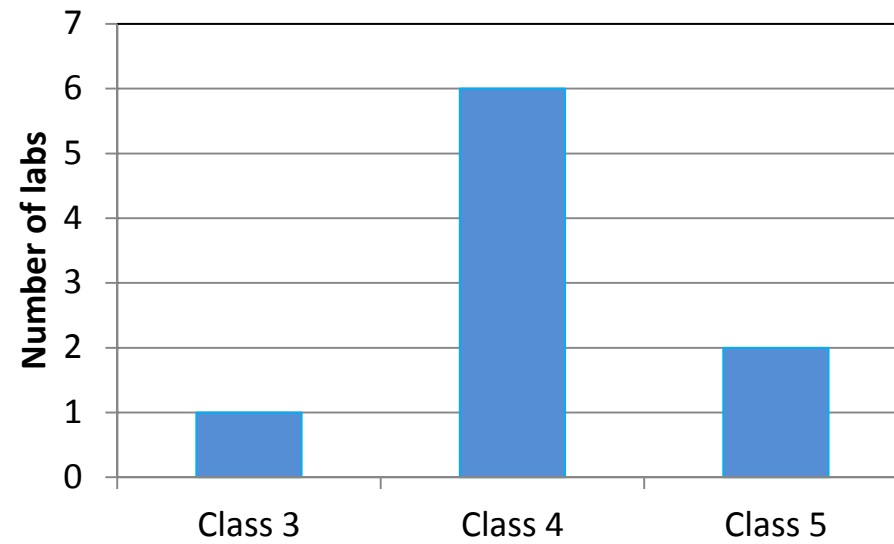
Marcus Arguello (dob 12/09/1987) is presenting with neurodegeneration with brain iron accumulation. Your local consultant neurologist has requested testing for this presentation and results are detailed in the table below.



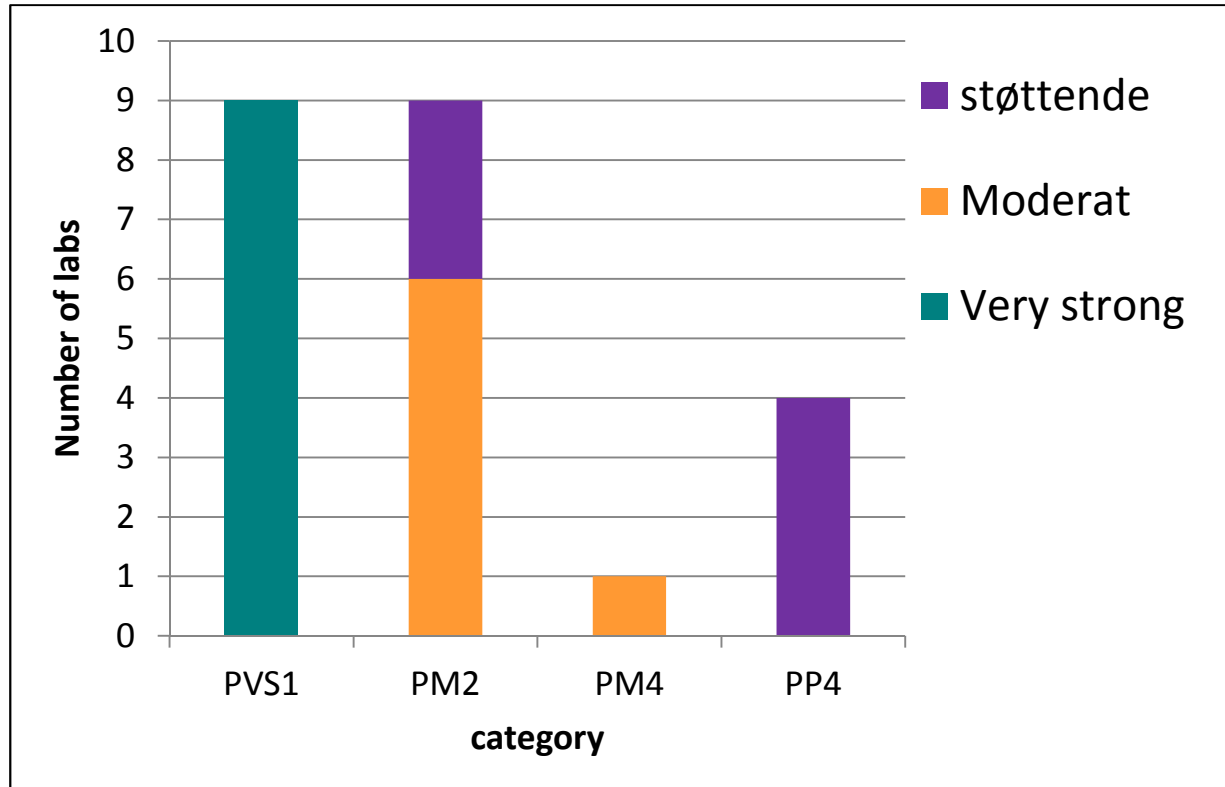
**C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter) heterozygous
PANK2 (NM_153638.3) c.377G>C (p.Gly126Ala) heterozygous**

C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter) heterozygous

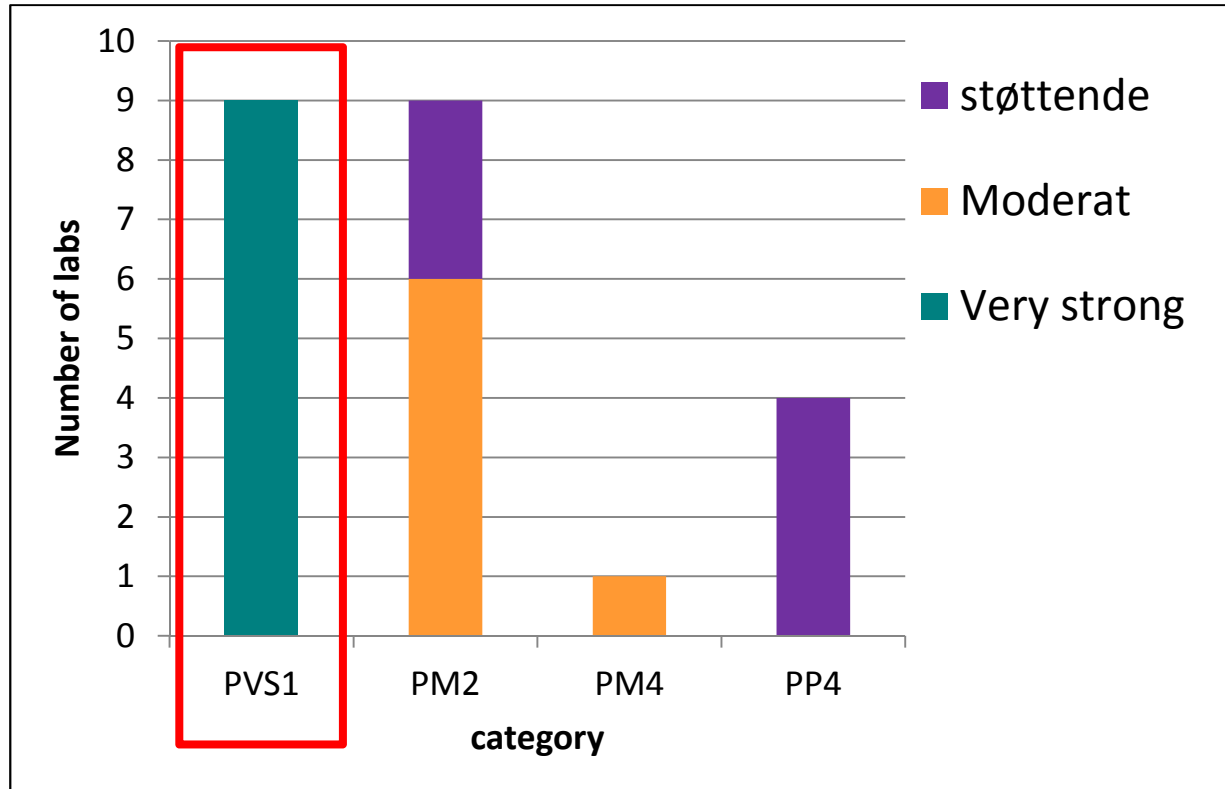
C19orf12 – autosomal recessive inherited neurodegeneration with brain iron accumulation 4 (OMIM#614298)



C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)



C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)

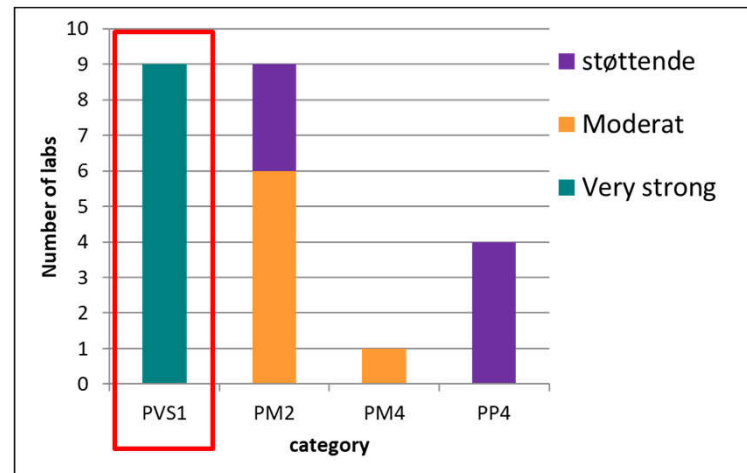


C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)

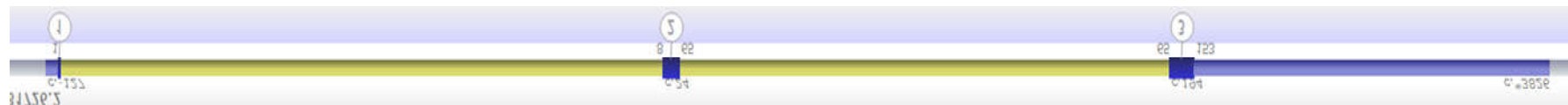
PVS1 - null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

Warnings:

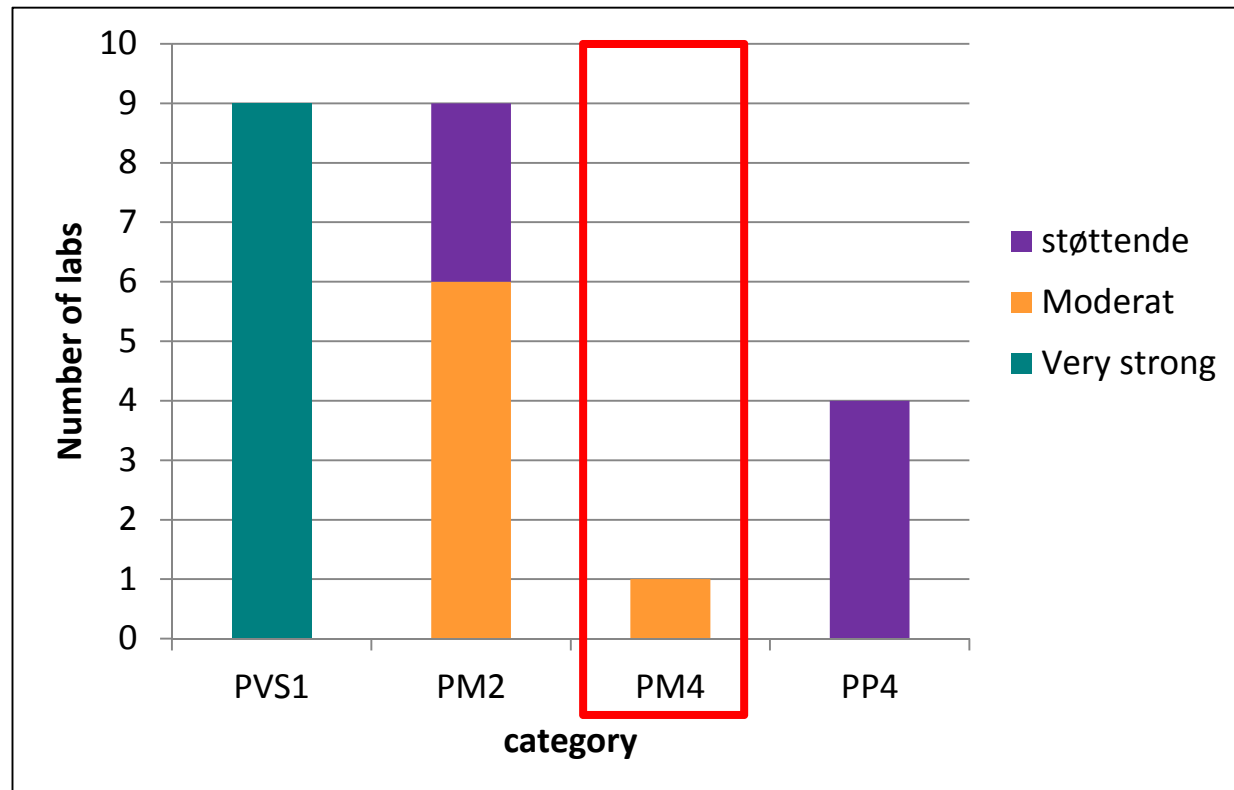
- Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3' end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts



| Labs | Evidens |
|------------|--|
| 1, 2, 7, 8 | null variant |
| 3, 4 | null variant, LOF known to be pathogenic |
| 5, 6, 9 | null variant, LOF known to be pathogenic Last exon (NMD negative) other C-terminal null variants reported |



C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)

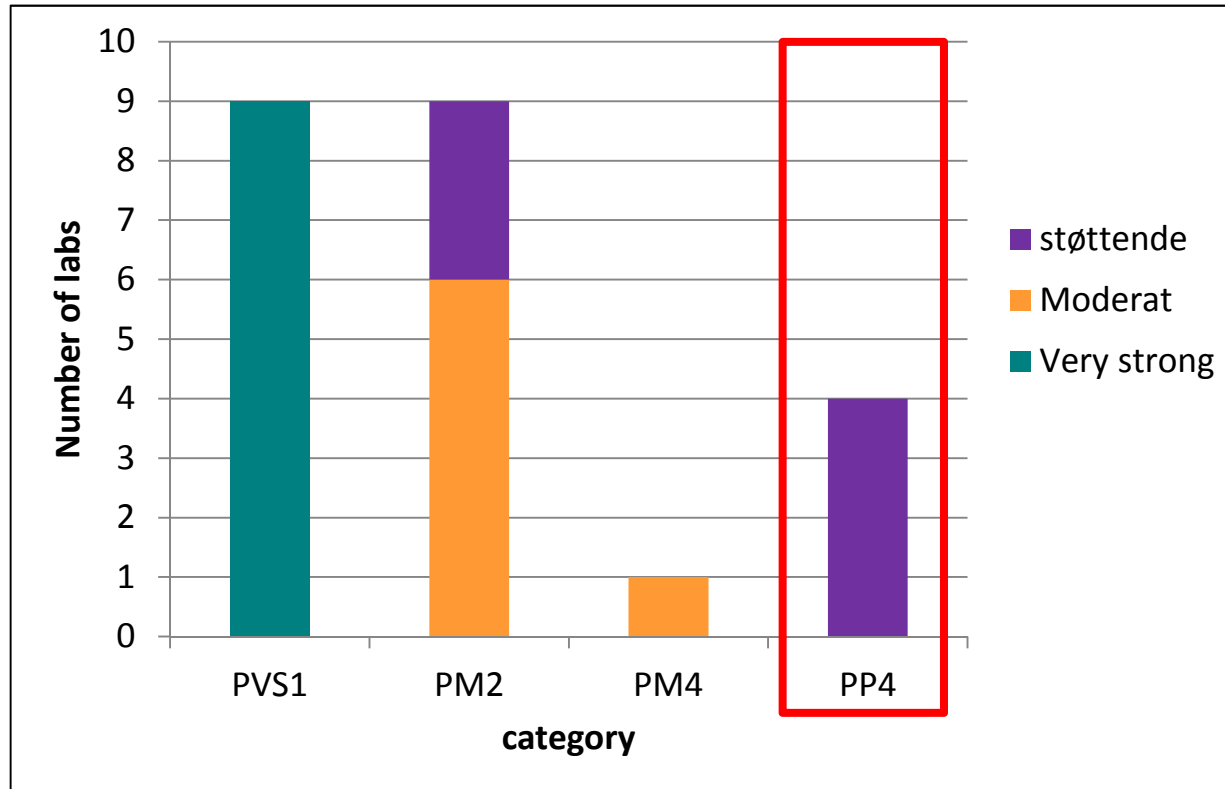


PM4 - Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants (152 aa → 89 aa)

PVS1 – null variant (insertion of stopcodon)
PM4 – protein length changes

»double counting«.

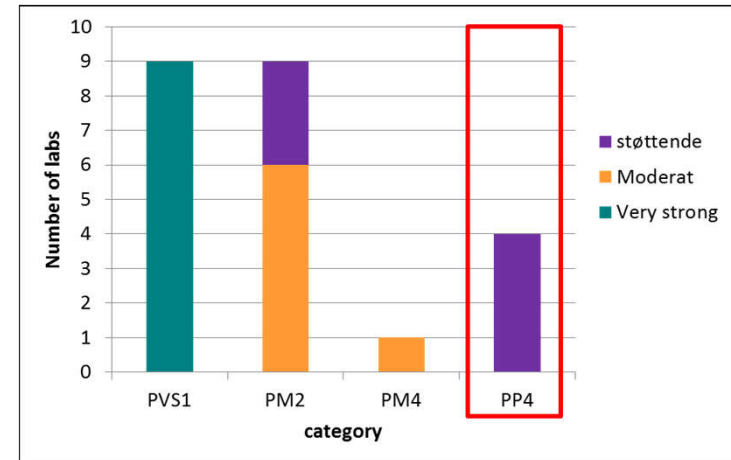
C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)



C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter)

PP4 - Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

Marcus Arguello (dob 12/09/1987) is presenting with neurodegeneration with brain iron accumulation. C19orf12 – autosomal recessive neurodegeneration with brain iron accumulation 4 (OMIM#614298)



OMIM: Neurodegeneration with brain iron accumulation - [PS234200](#) - 7 Entries

| Location | Phenotype | | Phenotype MIM number | Gene/Locus | Gene/Locus MIM number |
|--------------------------|--|-----|------------------------|--------------------------|------------------------|
| 17q21.2 | Neurodeg. with brain iron accu. 6 | AR | 615643 | COASY | 609855 |
| 19q12 | Neurodeg. with brain iron accu. 4 | AR | 614298 | C19orf12 | 614297 |
| 19q13.33 | Neurodeg. with brain iron accu. 3 | AD | 606159 | FTL | 134790 |
| 20p13 | Neurodeg. with brain iron accu. 1 | AR | 234200 | PANK2 | 606157 |
| 22q13.1 | Infantile neuroaxonal dystrophy 1 | AR | 256600 | PLA2G6 | 603604 |
| 22q13.1 | Neurodeg. with brain iron accu. 2B | AR | 610217 | PLA2G6 | 603604 |
| Xp11.23 | Neurodeg. with brain iron accu. 5 | XLD | 300894 | WDR45 | 300526 |

C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter) heterozygous

Likely Pathogen (Class 4)

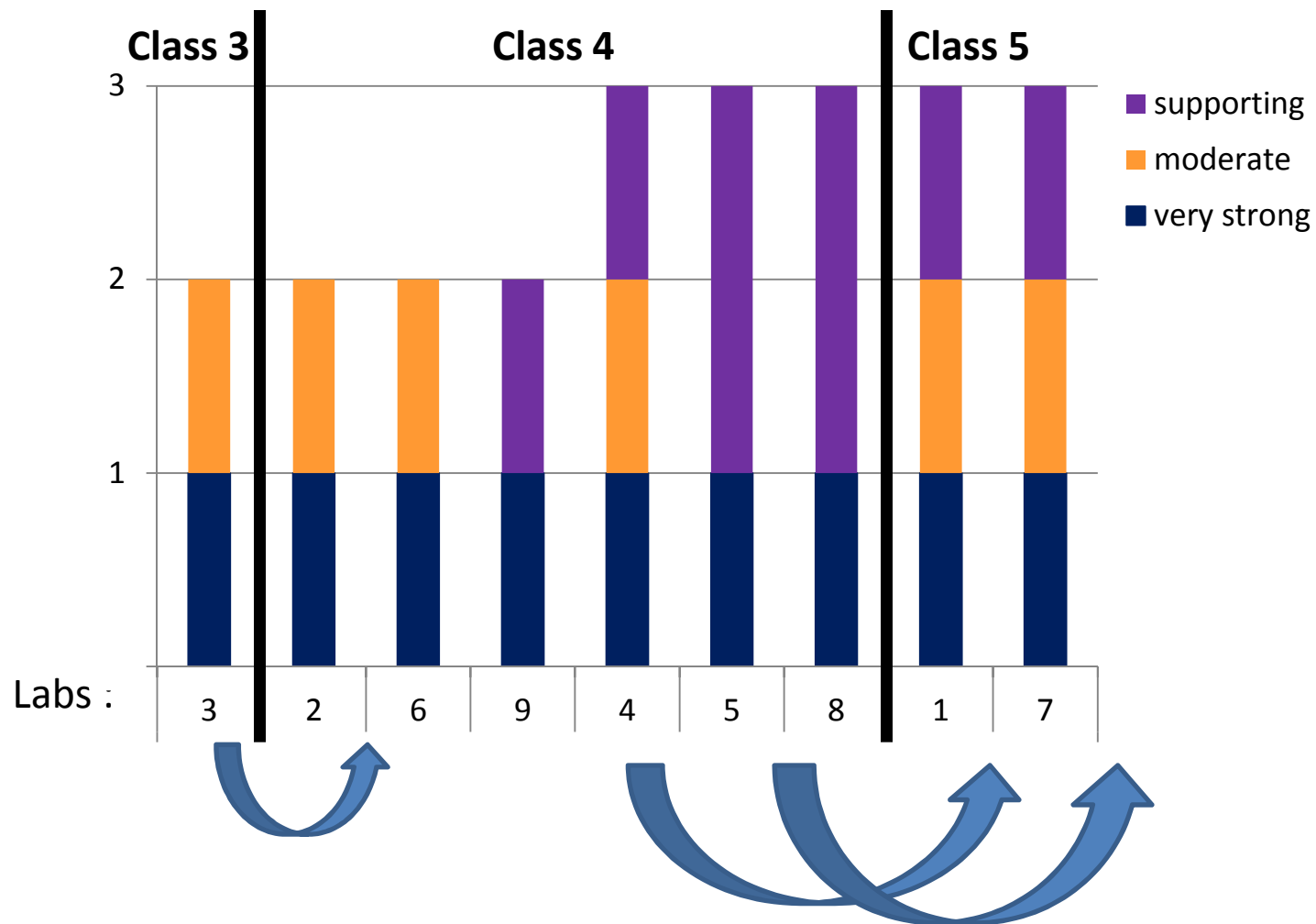
1 very strong + 1 moderate

Pathogen (Class 5)

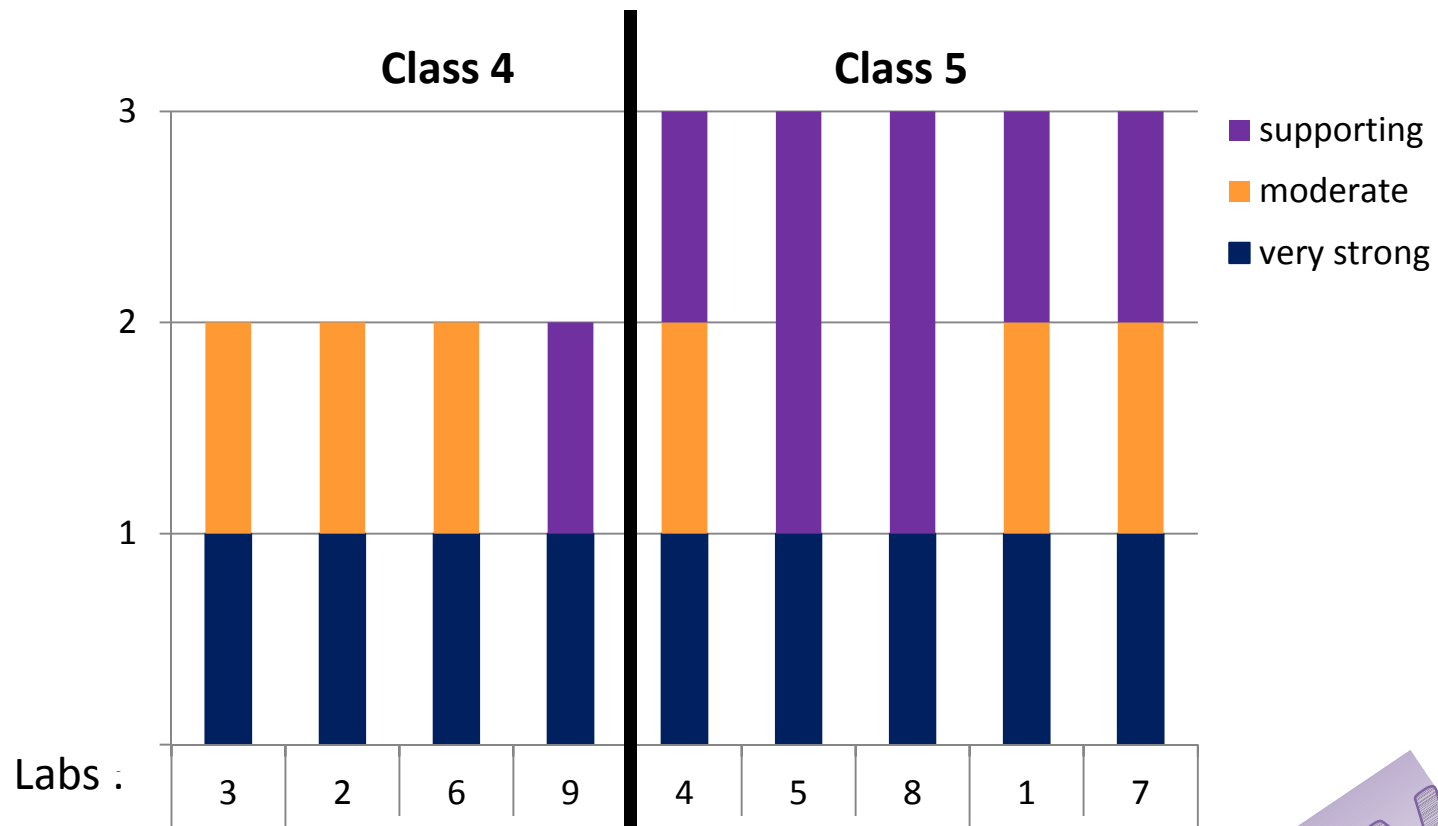
1 very strong + ≥ 2 moderate

+ 1 moderate + 1 supporting

+ ≥ 2 supporting




C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter) heterozygous



Why?

Patient 2

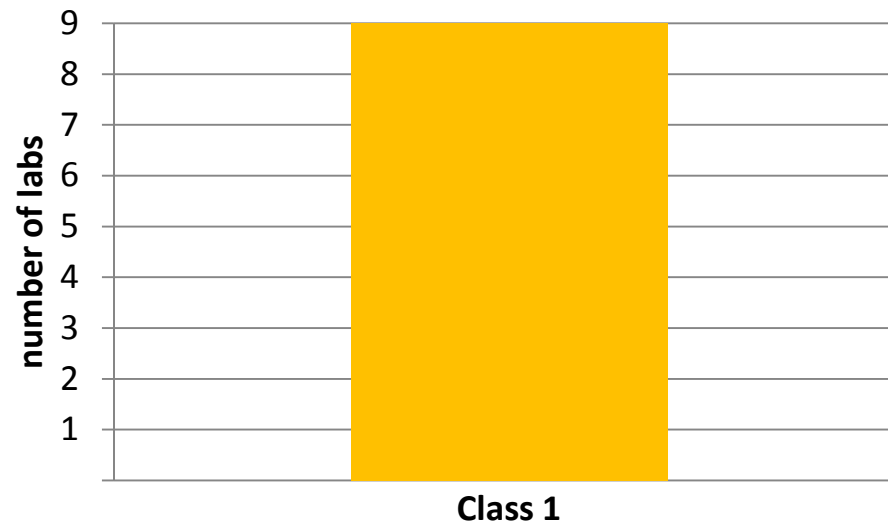
Marcus Arguello (dob 12/09/1987) is presenting with neurodegeneration with brain iron accumulation. Your local consultant neurologist has requested testing for this presentation and results are detailed in the table below.



C19orf12 (NM_001031726.2) c.268G>T (p.Glu90Ter) heterozygous
PANK2 (NM_153638.3) c.377G>C (p.Gly126Ala) heterozygous

PANK2 (NM_153638.3) c.377G>C (p.Gly126Ala) heterozygous


PANK2 – autosomal recessive Harp syndrome (OMIM#607236) and
autosomal recessive neurodegeneration with brain iron accumulation 1 (OMIM#234200)



BA1 - Allele frequency is >5% in GnomAd

Patient 3

John Howlett (dob 20/01/1985) and Elizabeth Howlett (dob 07/10/1984) have a son, James Howlett (dob 15/09/2015), who is presenting with signs of atypical Cornelia de Lange Syndrome (CdLS) including microcephaly, cleft palate and facial dysmorphism. John and Elizabeth are both healthy and are considering having another child. A Consultant Clinical Geneticist has requested testing of James and his parents. Sequence analysis of a panel of genes associated with CdLS and CdLS-like phenotypes was performed and James' results are detailed in the table below. Sanger sequencing did not detect the *NIPBL* variant in either parent.



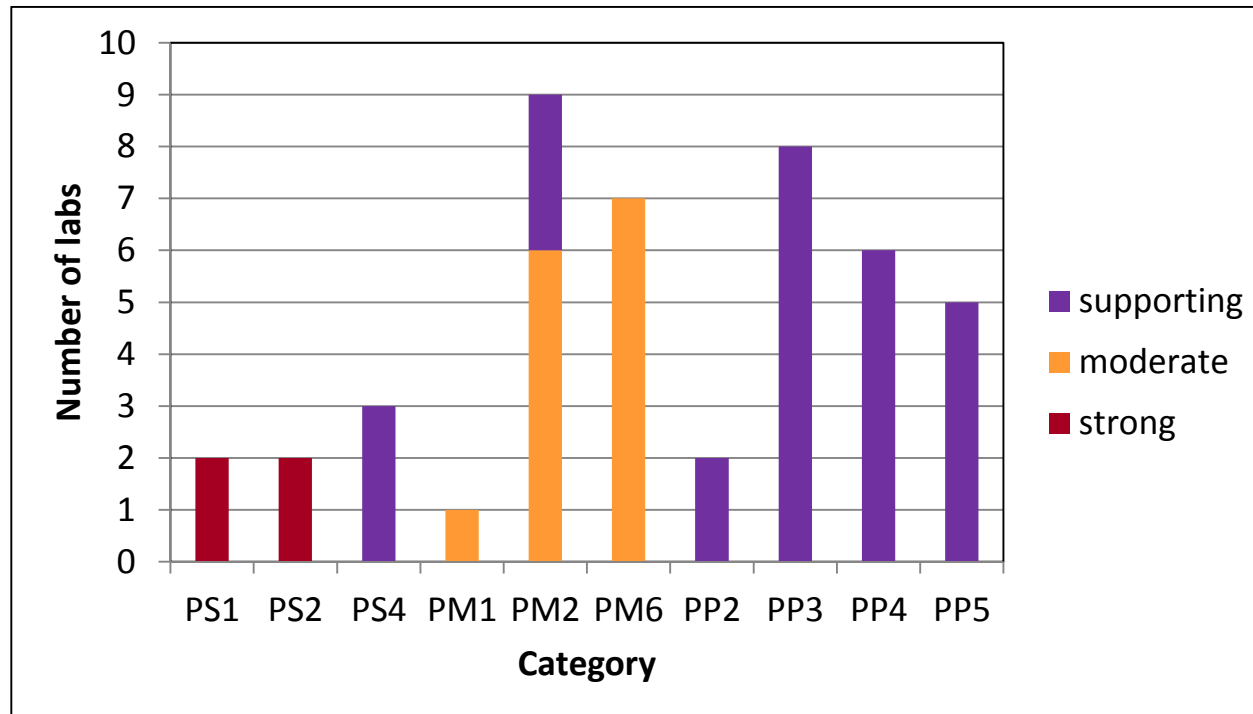
NIPBL (NM_133433.3) c.5594G>C (p.Arg1865Thr) *de novo*

NIPBL (NM_133433.3) c.5594G>C (p.Arg1865Thr) *de novo*

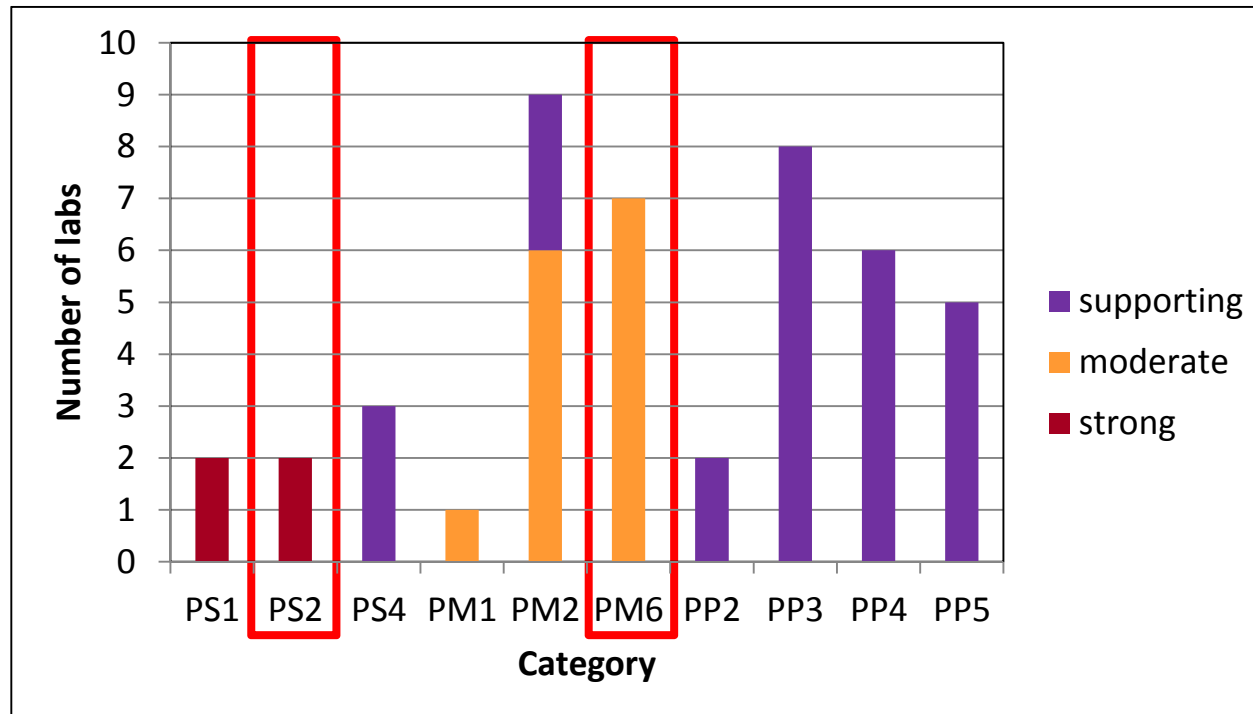
NIPBL – autosomal dominant Cornelia de Lange syndrome 1 (OMIM#122470)

All 9 labs suggested class 4!!!

NIPBL (NM_133433.3) c.5594G>C (p.Arg1865Thr) de novo



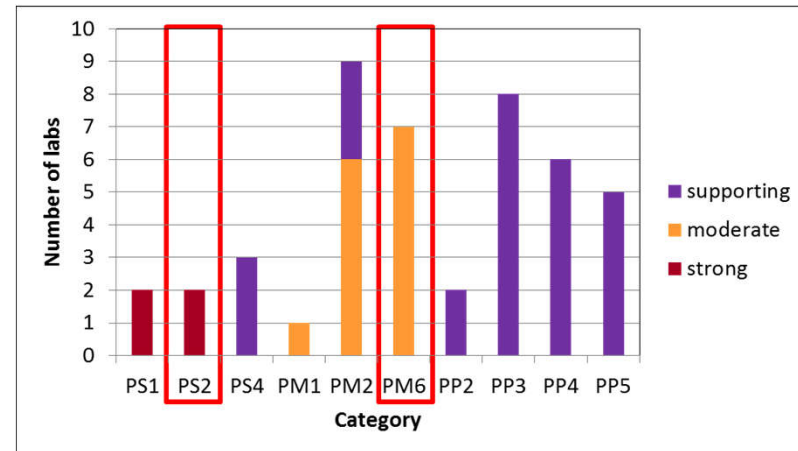
NIPBL (NM_133433.3) c.5594G>C (p.Arg1865Thr) de novo



NIPBL (NM_133433.3) c.5594G>C (p.Arg1865Thr) de novo

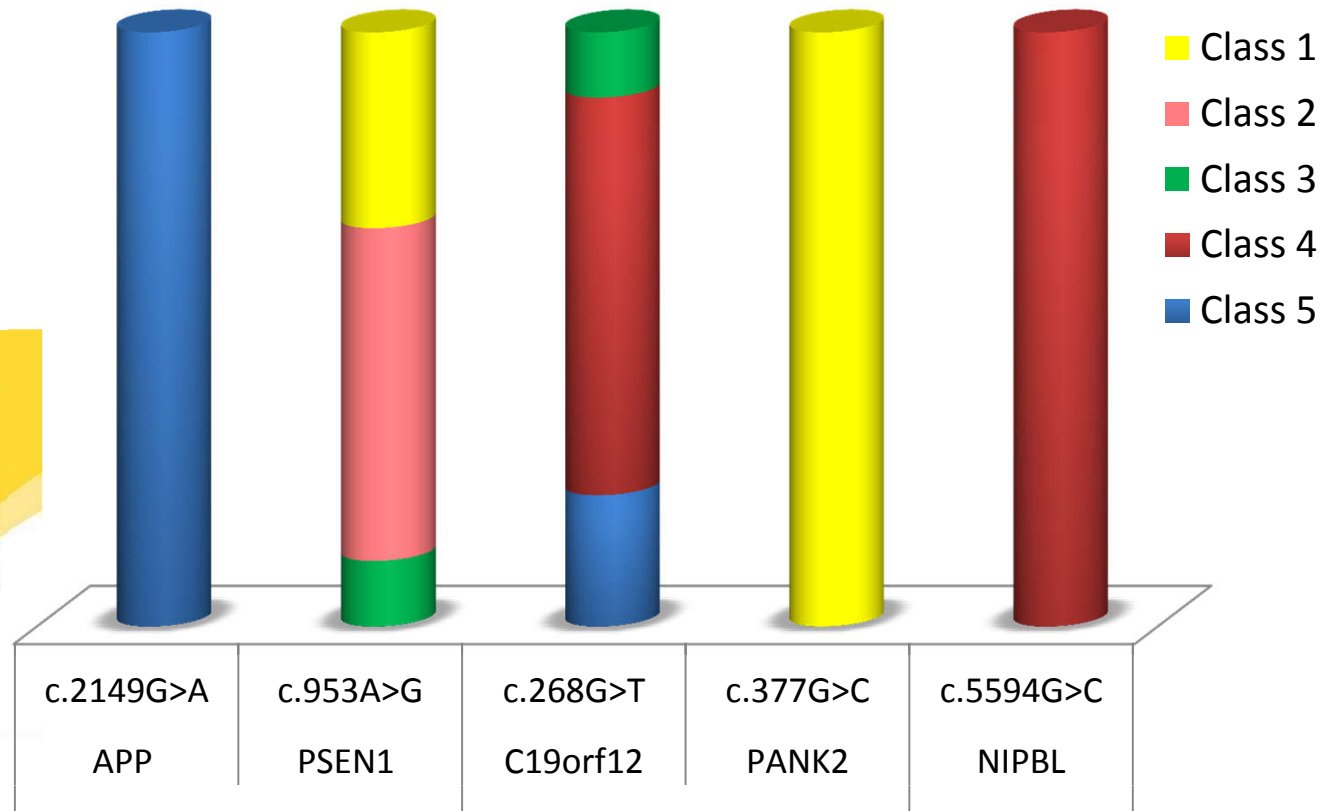
PS2 - *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

PM6 - Assumed *de novo*, but without confirmation of paternity and maternity



Summary

| Laboratorium | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------|----------|-----------|---|---|---|---|---|---|---|---|---|
| Patient 1 | APP | c.2149G>A | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | PSEN1 | c.953A>G | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 3 | 1 |
| Patient 2 | C19orf12 | c.268G>T | 5 | 4 | 3 | 4 | 4 | 4 | 5 | 4 | 4 |
| | PANK2 | c.377G>C | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Patient 3 | NIPBL | c.5594G>C | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |



Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium
Amendola et al., 2016

| Rule | Description | Clarifications and/or Suggestions |
|-------------|---|---|
| PS1 | variant with the same amino acid change as a previously established pathogenic variant, regardless of nucleotide change | does not include the same variant being assessed because it is not yet pathogenic, and the rule is intended for variants with a different nucleotide change |
| PM1 | variant located in a mutational hotspot and/or critical and well-established functional domain | more clarification is needed for applying this rule |
| PM4 | protein-length-changing variant | applicable for in-frame deletions, insertions, or stop-loss variants, but not frameshifts, nonsense, and splice variants |
| PM5 | novel missense variant at amino acid with different pathogenic missense change | ensure pathogenicity of previously reported variant |
| | | suggest changing “novel” to “different” because some variants that are not novel might require assessment with this rule |
| PP4 | the patient’s phenotype or family history is highly specific to the genotype | not meant to be used for genetically heterogeneous conditions or conditions with unsolved etiology |
| PP5, BP6 | variant called pathogenic or benign by a reputable source | only applicable when evidence is not available (e.g., Sharing Clinical Reports Project) |

Should the ACMG Guidelines be adopted as national guidelines for variant classification in Norway?

- Training (workshops)
- External quality assessment
- Sharing classification
- Gene/disease specific criteria (ClinGen resource consortium)