Commonwealth Medicine

Clinical Delivery and Informatics Solutions

Variant Interpretation, Tracking, and Reporting

Presented for:

Molecular Training Workshop

CDC, Atlanta, GA

Presented by:

Anne Marie Comeau, PhD

Deputy Director,
New England Newborn Screening Program
Professor of Pediatrics,
University of Massachusetts Medical School

February 2020

Preparations to “Go Live” Simultaneously

X-ALD
Pompe
MPS-1
(and SMA)

Collegial Learning

Newborn Screening for
Adrenoleukodystrophy, Pompe Disease and
Mucopolysaccharidosis Type I:
A Hands-On Workshop
At the
NYS Newborn Screening Program

July 19-21, 2017

ACMG Guidelines for Sequence Variations

ACMG Guidelines for Sequence Variations

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

GENETICS in MEDICINE | Volume 17 | Number 5 | May 2015

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

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Classification criteria for pathogenic variants

Evidence of Pathogenicity	Category	Definition
VERY STRONG (PVS)	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** ➢ 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
Strong (PS1-PS4)	PS1	**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 ➢ Beware of changes that impact splicing rather than at the amino acid/protein level
	PS2	**De novo (both maternity and paternity confirmed) in a patient with the disease and no family history** NOTE: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.
	PS3	**Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product** 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.
	PS4	**The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls** 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 guidance. 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-PM6)	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**
	PM2	**Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium** ➢ Population data for insertions/deletions may be poorly called by next-generation sequencing.
	PM3	**For recessive disorders, detected in trans with a pathogenic variant** Note: This requires testing of parents (or offspring) to determine phase.
	PM4	**Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants**
	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 ➢ Beware of changes that impact splicing rather than at the amino acid/protein level.
	PM6	**Assumed de novo, but without confirmation of paternity and maternity**
Supporting (PP1-PP5)	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.)** ➢ 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.
	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**

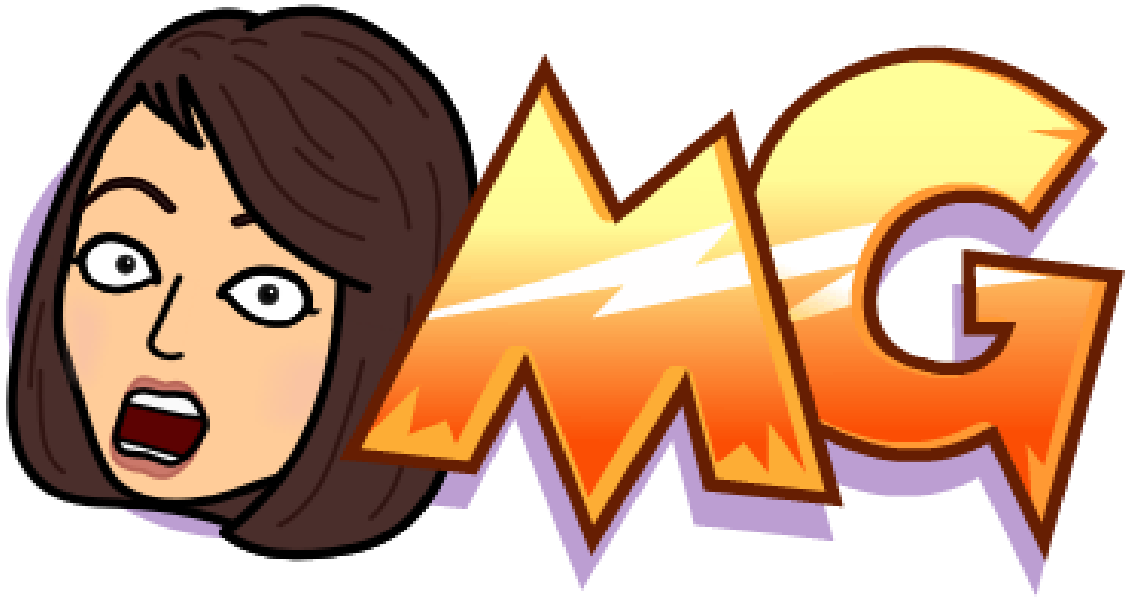
Classification criteria for benign variants

Evidence of Benign Impact	Category	Definition
Stand-alone (BA1)	BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
Strong (BS1-BS4)	BS1	Allele frequency is greater than expected for disorder (see Table 6)
	BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.
	BS3	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing
	BS4	Lack of segregation in affected members of a family Caveat: The presence of phenocopies for common phenotypes (i.e., cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.
Supporting (BP1-BP7)	BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease
	BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern
	BP3	In-frame deletions/insertions in a repetitive region without a known function
	BP4	Multiple line of computational evidence suggests no impact on a gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.
	BP5	Variant found in a case with an alternative molecular basis for disease
	BP6	Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation
	BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence not the creation of a new splice site AND the nucleotide is not highly conserved

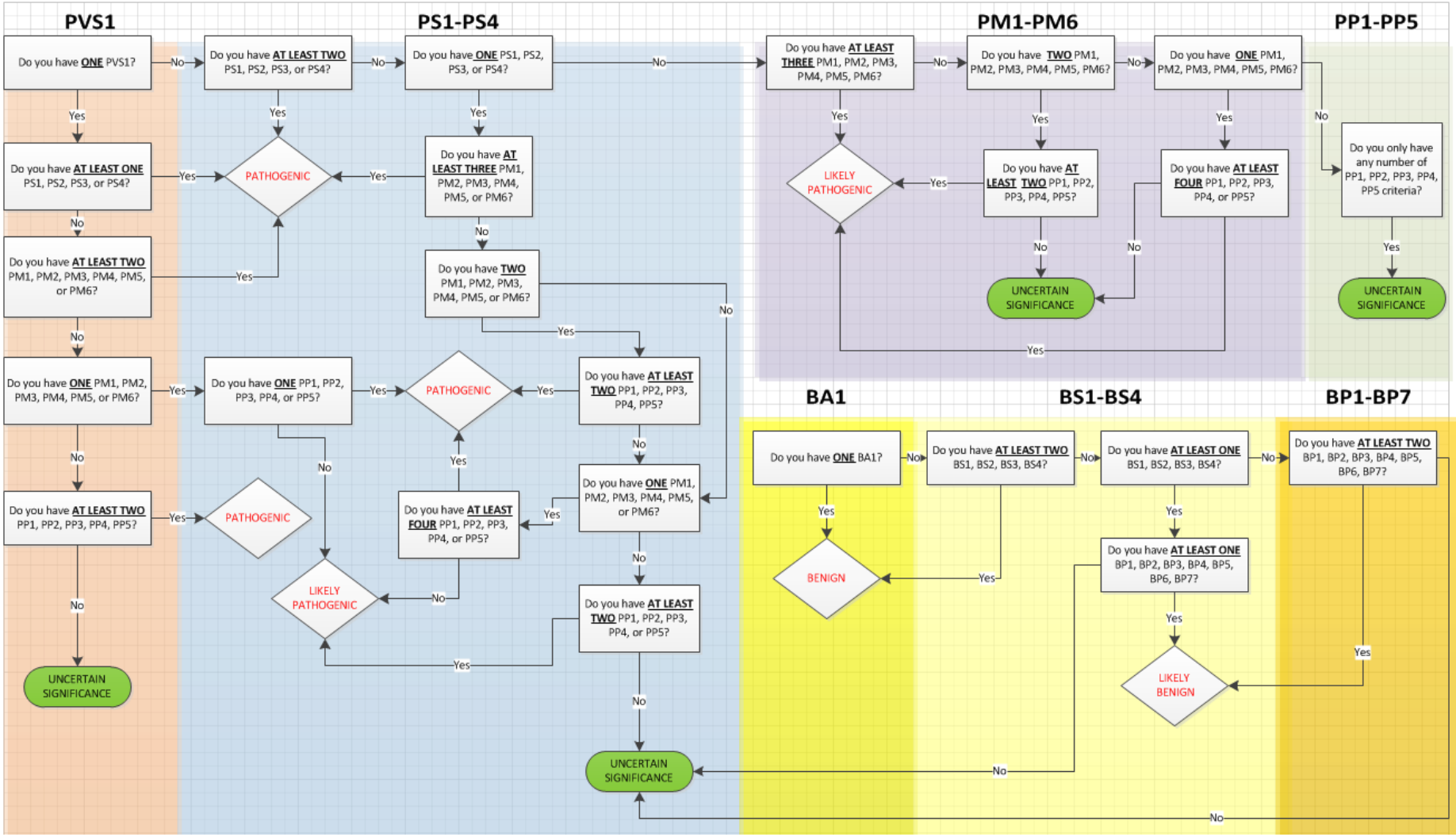
ACMG Rules for Combining Criteria

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)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory



UMass Flow Chart for Classification

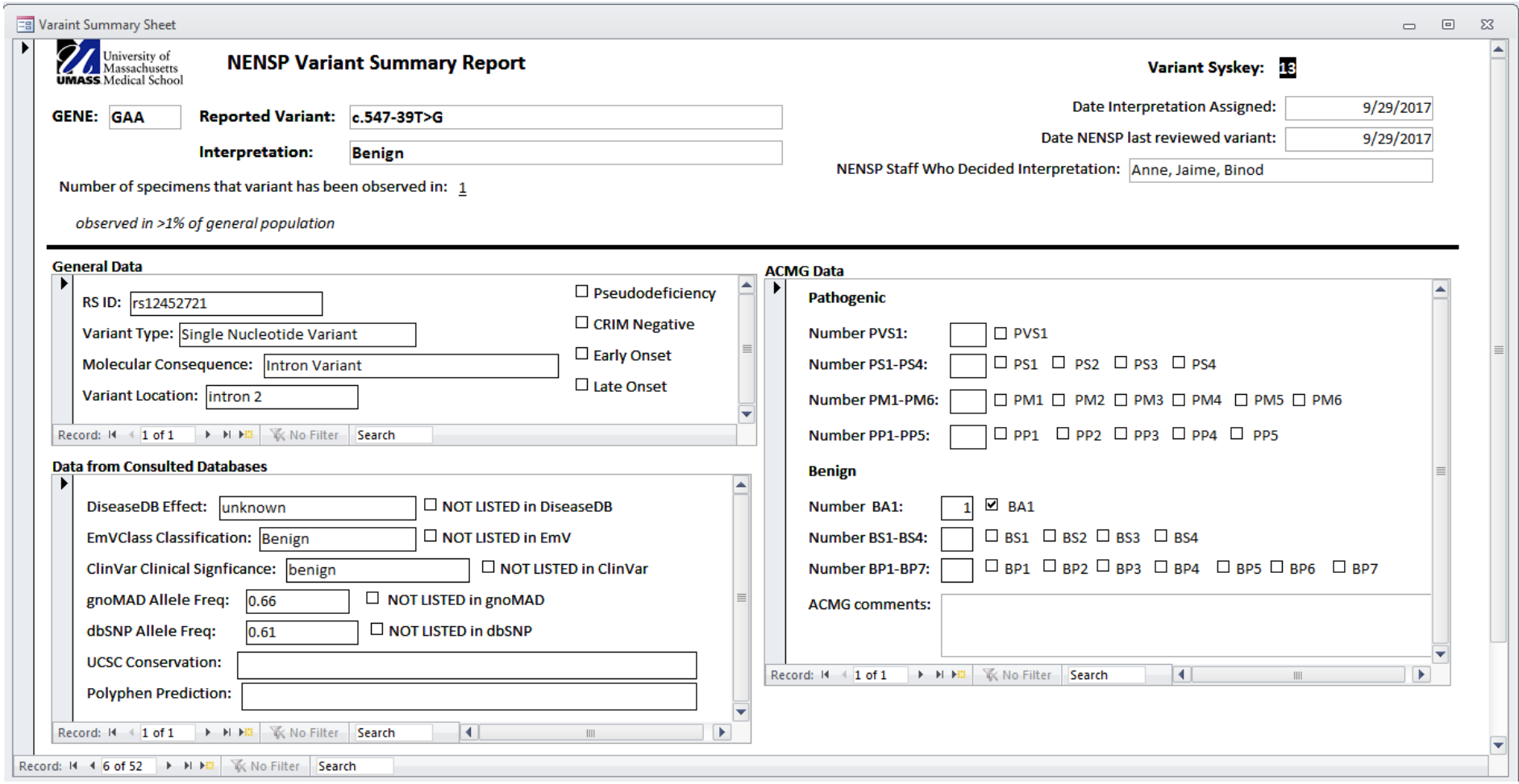


START NOW !!!

Do You Have a Variant Database? It will help you to:

- Track the information you collect
- Help you to determine interpretation
- Use as the foundation for your report

Variant Example – c.547-39T>G



VARIANT HGVS NAME: c.547-39T>G Date of Evaluation: 10/20/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant**
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	none
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Intron 2
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	
Effect:	unknown
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	Exon 3
Classification	Benign
Date the variant was last reviewed.	8/7/17
Comment:	BP6

6. **Broad Genome Aggregation Database (gnomAD)** http://gnomad.broadinstitute.org/
   Effective use of this database requires use of the .csv file before use of the online database.
   a. Input the gene name
   b. Double click on the "Export table to CSV" - (this is an action that should be done daily, or each time a search is begun in case the database is updated).
   c. Use control F in the excel file to find your variant.
   d. Once you have found your variant, copy the rs id and use the rs id in the search of the online database (again, use the control F function). When you have found your variant in the online database, click on the rs id link and this will provide essential links to ClinVar, dbSNP, etc.

Listed?	yes
Rs id (may be in column one or csv file)	rs12452721
Consequence variant name, optional :	
Annotation:	intron
Allele freq:	0.66
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment:	BA1

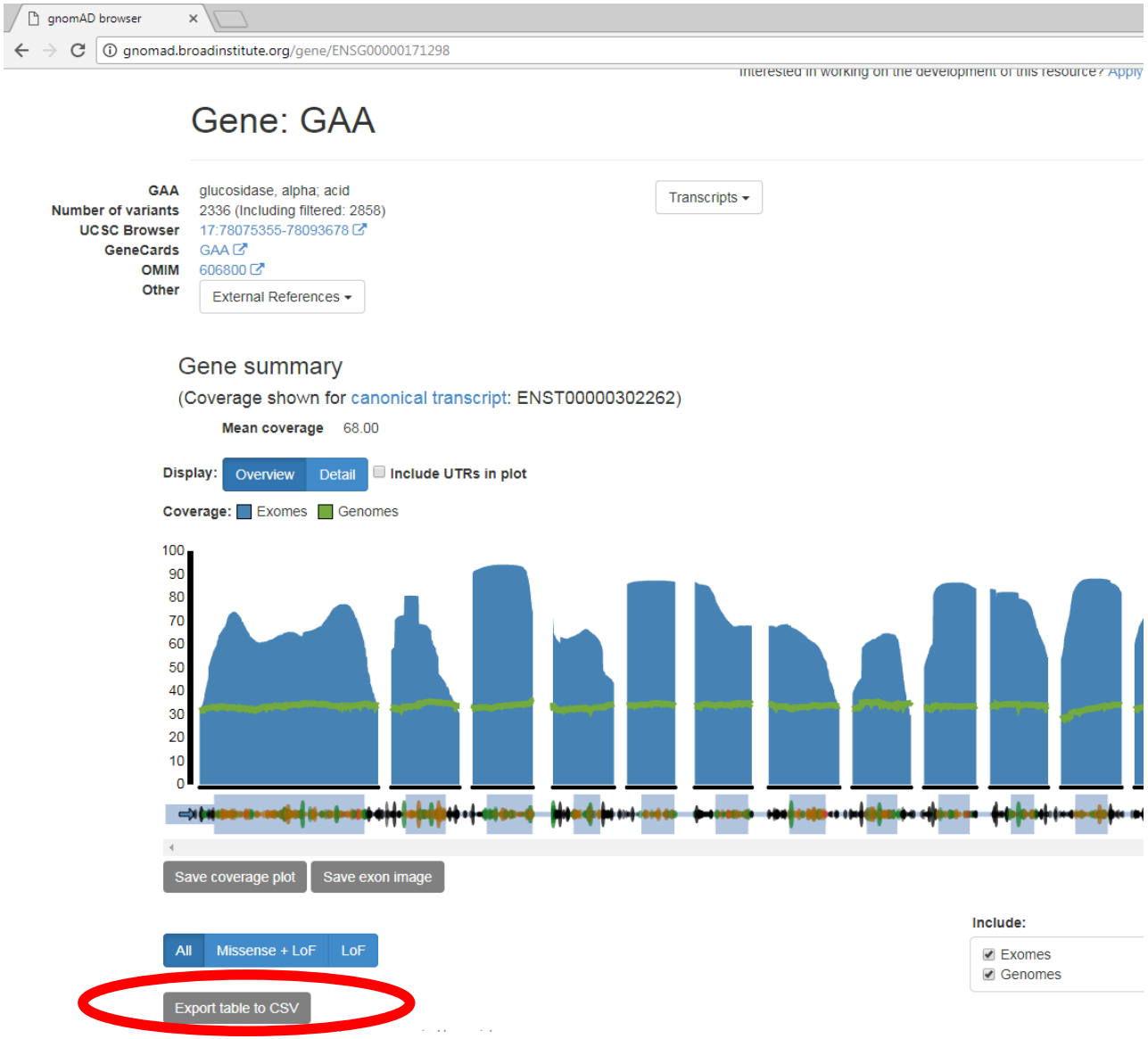
7. **Broad Exome Aggregation Database (ExAC)** http://exac.broadinstitute.org/ SKIP THIS IF YOU GOT INFO FROM GNOMAD

Listed?	
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment	

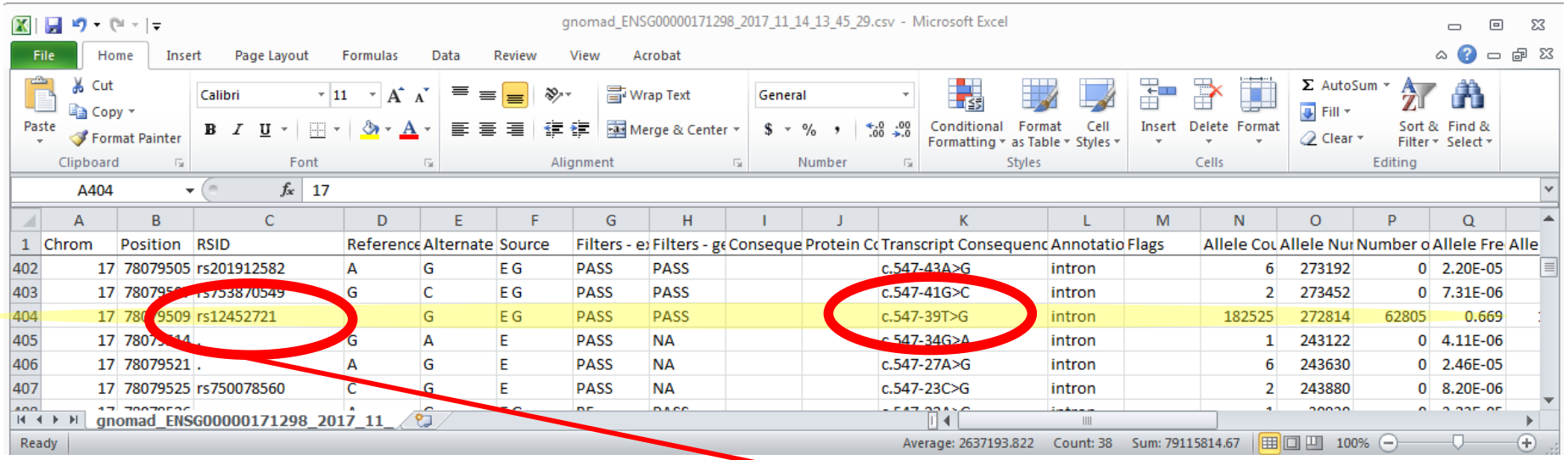
8. **Clin Var** (*may be easily accessed through gnomad link if you found an rs id*) www.ncbi.nlm.nih.gov/clinvar/

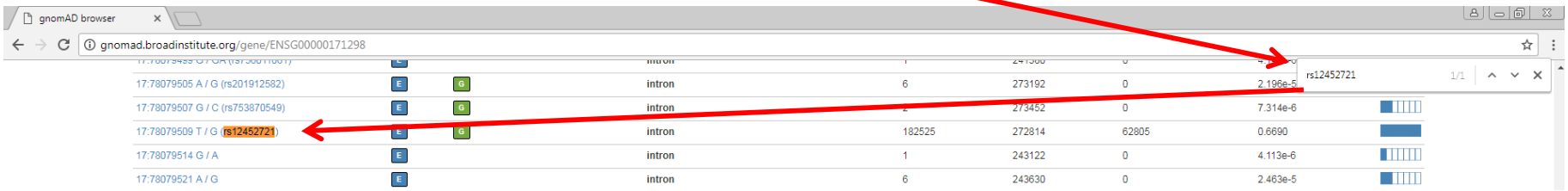
Listed?	yes
Clinical significance	benign
Last evaluated	No date
Variant Type	SNV
Rs id	12452721
Molecular consequence	Intron variant
Notes on the number of non-EMV submissions to Clin Var (EMV data noted above)	Benign by one non-emv report
Comment	BP6

gnoMAD



gnoMAD





gnoMAD

Interested in working on the development of this resource? Apply here.

Variant: 17:78079509 T / G

	Exomes	Genomes	Total
Filter	Pass	Pass	
Allele Count	161943	20582	182525
Allele Number	241986	30828	272814
Allele Frequency	0.6692	0.6676	0.6690
dbSNP	rs12452721		
UCSC	17-78079509-T-G		
ClinVar	Click to search for variant in Clinvar		

Genotype Quality Metrics

Site Quality Metrics

Report this variant

Annotations

This variant falls on 5 transcripts in 1 genes:

intron

- GAA Transcripts

Note: This list may not include additional transcripts in the same gene that the variant does not overlap.

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Ashkenazi Jewish*	7871	10114	3058	0.7782
European (Finnish)	17134	22666	6462	0.7559
European (Non-Finnish)	93110	125876	34436	0.7397
Other	4552	6418	1634	0.7093
South Asian	20822	30744	7130	0.6773
African	12811	23872	3405	0.5367
East Asian	9984	18790	2640	0.5313
Latino	16241	34334	4040	
Total	**182525**	**272814**	**62805**	**0.6690**

Include: Exomes Genomes

New England Newborn Screening Program
University of Massachusetts Medical School

VARIANT HGVS NAME: c.547-39T>G Date of Evaluation: 10/20/17

9. **dbSNP** (access through gnomad link, which is best, or clin var, which means you should go through the web page's rs id to get to the best presentation of data) https://www.ncbi.nlm.nih.gov/projects/SNP/

Listed?	yes
Allele freq:	0.61
Source of allelic frequency	1000 genomes
Comment:	**BA1**

10. **UCSC genome browser** (accessed through gnomad link)

Listed?	
Conservation?	
Comment	

11. **polyphen for use with amino acid changes only** http://genetics.bwh.harvard.edu/pph2/

12. **Other sites of desperation: google scholar etc….sites that may be of use**

VARIANT HGVS NAME: c.547-39T>G Date of Evaluation: 10/20/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant** BP6 BA1 BENIGN
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	none
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Intron 2
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	
Effect:	unknown
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	Exon 3
Classification	Benign
Date the variant was last reviewed.	8/7/17
Comment:	BP6

Variant Example – c.60G>A

New England Newborn Screening Program
University of Massachusetts Medical School

VARIANT HGVS NAME c.60G>A _p.Ala20= Date of Evaluation: November 7, 2017

Variant Assessment Worksheet

1. **Condition: MPS-I Variant**
2. **Reviewed by NENSP before? no**
3. **Other newborn screening program contribution**

Name of program	**no**
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) ***If you can get to the point of finding a good rs number (see below), you are in good shape.***

4. **MPS-1 database (DSdb)**
   http://mps1-database.org/mutants/

   this is NOT a great database – but it's a start.
   - *Click on "list"*
   - *Copy list each time into excel and sort to find variant or try Control F.*
   - *Available database fields are listed in first set below (I would copy the record onto this sheet):*

id	**Not listed**
locus	
Mutation type	
genotype	
phenotype	
race	
author	
paper	
Abstracts supporting pathogenic:	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

Variant Example – c.60G>A

VARIANT HGVS NAME c.60G>A _p.Ala20= Date of Evaluation: November 7, 2017

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	
Location *note that intronic variants seem to be listed by closest exon*:	**EXON 1**
Classification	**BENIGN**
Date the variant was last reviewed.	**10/7/2014**
Comment:	**BP6**

6. **Broad Genome Aggregation Database (gnomAD)** http://gnomad.broadinstitute.org/

Effective use of this database requires use of the .csv file before use of the online database.

a. Input the gene name
b. Double click on the "Export table to CSV" - (this is an action that should be done daily, or each time a search is begun in case the database is updated).
c. Use control F in the excel file to find your variant.
d. Once you have found your variant, copy the rs id and use the rs id in the search of the online database (again, use the control F function). When you have found your variant in the online database, click on the rs id link and this will provide essential links to ClinVar, dbSNP, etc.

Listed?	**no**
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment:	**Using rsid from clin var takes me to a missense mutation and indicates original codon of arg. But I also see ala. Missense must be for another transcript.**

7. **Broad Exome Aggregation Database (ExAC)** http://exac.broadinstitute.org/ SKIP THIS IF YOU GOT INFO FROM GNOMAD

Listed?	
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment	

Variant Example – c.60G>A

VARIANT HGVS NAME c.60G>A _p.Ala20= Date of Evaluation: November 7, 2017

8. **Clin Var** (*may be easily accessed through gnomad link if you found an rs id*) www.ncbi.nlm.nih.gov/clinvar/

Listed?	
Clinical significance	benign
Last evaluated	Jun 14, 16
Variant Type	snv
Rs id	rs10902762
Molecular consequence	synonymous
Notes on the number of non-EMV submissions to Clin Var (EMV data noted above)	Two benign in addition to benign emv
Comment	

9. **dbSNP** (access through gnomad link, which is best, or clin var, which means you should go through the web page's rs id to get to the best presentation of data) https://www.ncbi.nlm.nih.gov/projects/SNP/

Listed?	yes
Allele freq:	0.39 BA1
Source of allelic frequency	by 1000G,by 2hit 2allele,by cluster,by frequency,by hapmap
Comment:	Noting variety of functions.

10. **UCSC genome browser** (accessed through gnomad link)

Listed?	
Conservation?	
Comment	

11. **polyphen for use with amino acid changes only** http://genetics.bwh.harvard.edu/pph2/

12. **Other sites of desperation: google scholar etc….sites that may be of use**

1000 genomes from clin var rsid: 39% BA1

Variant Example – c.60G>A

New England Newborn Screening Program
University of Massachusetts Medical School

VARIANT HGVS NAME c.60G>A _p.Ala20= Date of Evaluation: November 7, 2017

Variant Assessment Worksheet

1. **Condition: MPS-I Variant** bp6 BA1 **BENIGN**
2. **Reviewed by NENSP before? no**
3. **Other newborn screening program contribution**

Name of program	**no**
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

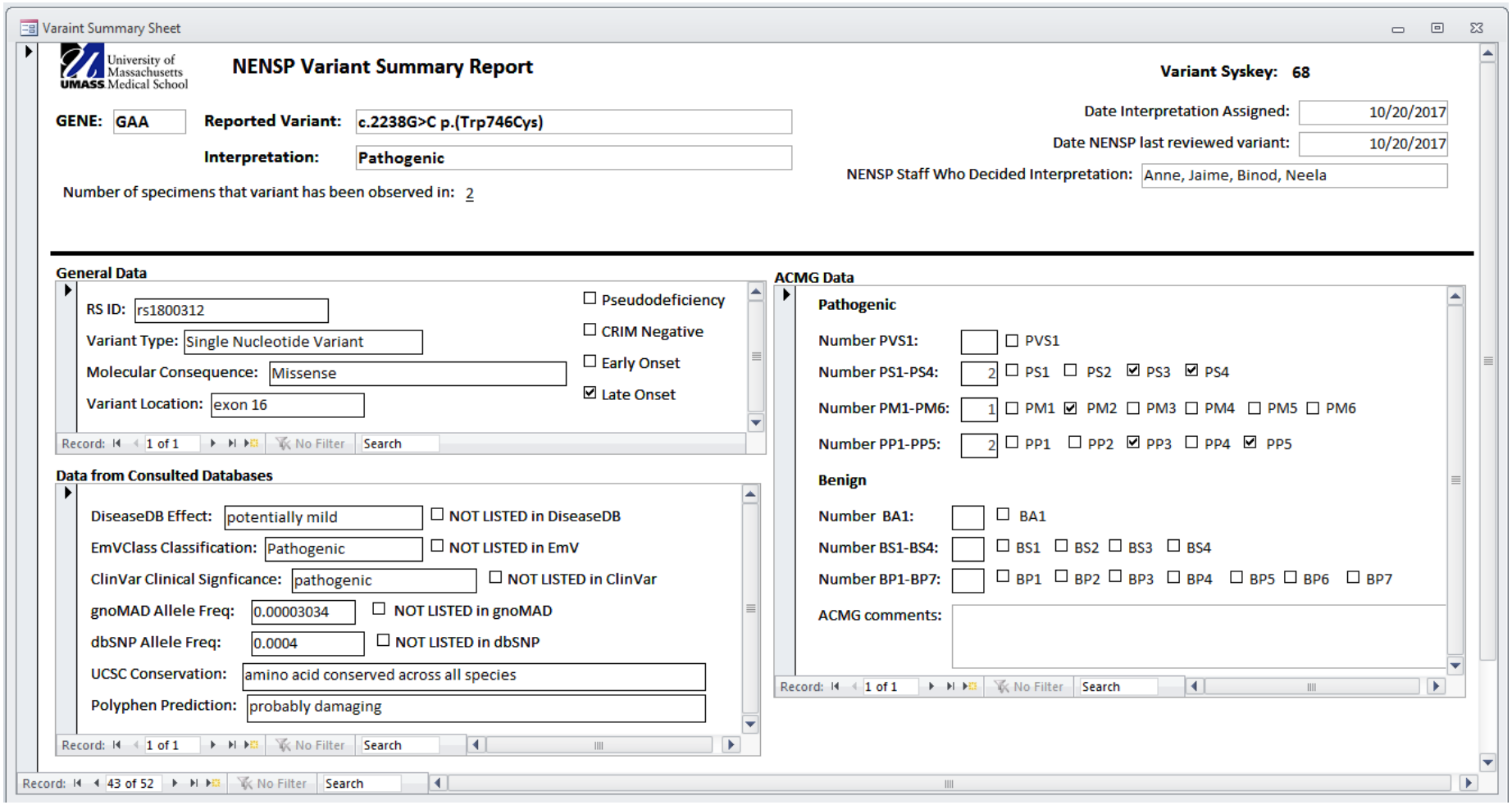
4. **MPS-1 database (DSdb)**
   http://mps1-database.org/mutants/

   this is NOT a great database – but it's a start.

   - *Click on "list"*
   - *Copy list each time into excel and sort to find variant or try Control F.*
   - *Available database fields are listed in first set below (I would copy the record onto this sheet):*

id	**Not listed**
locus	
Mutation type	
genotype	
phenotype	
race	
author	
paper	
Abstracts supporting pathogenic:	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

Variant Example – c.2238G>C p.(Trp746Cys)



Variant Example – c.2238G>C p.(Trp746Cys)

VARIANT HGVS NAME: c.2238G>C p.(Trp746Cys) Date of Evaluation: 10/16/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant**
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	No
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Exon 16
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	p.Trp746Cys
Effect:	potentially mild
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	pathogenic
Classification	
Date the variant was last reviewed.	6/15/17
Comment:	PP5

Pompe Database

MUTATIONS IN HUMAN

cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Updated: May 2016

MUTATIONS IN HUMAN ACID ALPHA-GLUCOSIDASE

Location	DNA HGVnomenclature	Protein HGVnomenclature	Effect	Year First	Link
exon16	c.2238G>C	p.Trp746Cys	potentially mild	1994	PubMed
exon16	c.2238G>A	p.Trp746*	very severe	2006	PubMed

EGL (EmV) Database

EGL Genetics | Home

www.egl-eurofins.com/emvclass/emvclass.php

Gene GAA Search

(Search HGNC)

You searched for: GAA

Pathogenic: 54 **Likely Pathogenic:** 8 **VOUS:** 189 **Likely Benign:** 11 **Benign:** 55

Total records returned: 318

You may further refine your search by entering exon, change nomenclature, or classification terms here

Show 100 records Search: 2238

Order	Gene	Exon	Nucleotide Change	Protein Change	Alias Listing	Classification	Last Reviewed	
262	GAA	Ex16	NM_000152.3:c.2238G>C	p.Trp746Cys | p.W746C	NM_000152.3:c.2238G>C, NM_001079803.1:c.2238G>C, NM_001079804.1:c.2238G>C, XM_005257193.1:c.2238G>C, XM_005257194.1:c.2238G>C	Pathogenic	06/15/2017	?
263	GAA	Ex16	NM_000152.3:c.2238G>A	p.Trp746* | p.W746X	LRG_673t1:c.2238G>A, NM_000152.3:c.2238G>A, NM_001079803.1:c.2238G>A, NM_001079804.1:c.2238G>A, XM_005257194.1:c.2238G>A	Pathogenic	01/17/2017	?

Showing 1 to 2 of 2 records (filtered from 318 total entries)

First Previous 1 Next Last

Variant Example – c.2238G>C p.(Trp746Cys)

VARIANT HGVS NAME: c.2238G>C p.(Trp746Cys) Date of Evaluation: 10/16/17

6. **Broad Genome Aggregation Database (gnomAD)** http://gnomad.broadinstitute.org/
   Effective use of this database requires use of the .csv file before use of the online database.
   a. Input the gene name
   b. Double click on the "Export table to CSV" - (this is an action that should be done daily, or each time a search is begun in case the database is updated).
   c. Use control F in the excel file to find your variant.
   d. Once you have found your variant, copy the rs id and use the rs id in the search of the online database (again, use the control F function). When you have found your variant in the online database, click on the rs id link and this will provide essential links to ClinVar, dbSNP, etc.

Listed?	yes
Rs id (may be in column one or csv file)	rs1800312
Consequence variant name, optional :	
Annotation:	missense
Allele freq:	0.0003034
Was there a warning about the allelic frequency – maybe insufficient data?	no
Comment:	PM2

7. **Broad Exome Aggregation Database (ExAC)** http://exac.broadinstitute.org/ SKIP THIS IF YOU GOT INFO FROM GNOMAD

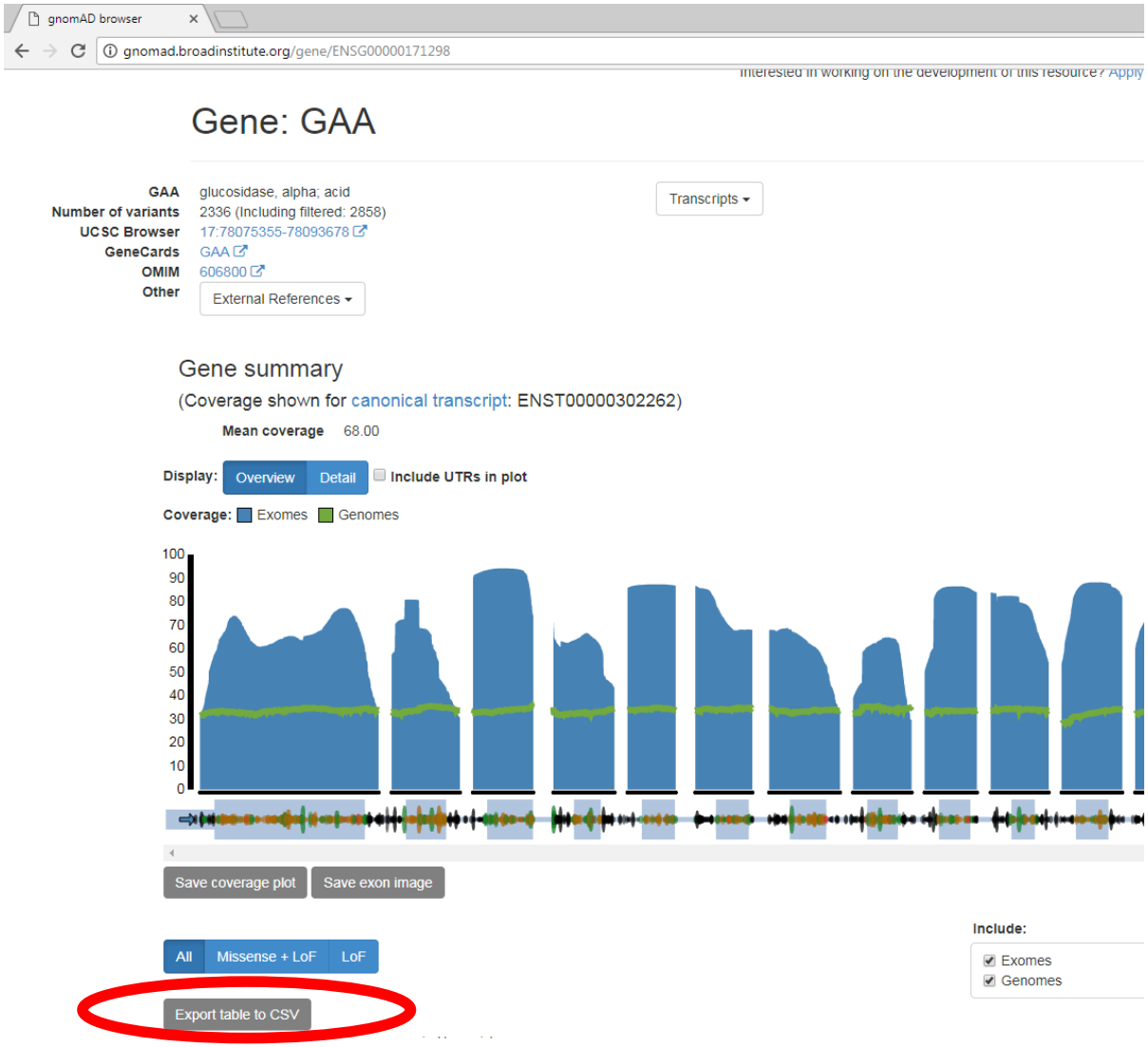
Listed?	
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment	

8. **Clin Var** (*may be easily accessed through gnomad link if you found an rs id*) www.ncbi.nlm.nih.gov/clinvar/

Listed?	yes
Clinical significance	pathogenic
Last evaluated	1/25/2017
Variant Type	SNV
Rs id	
Molecular consequence	missense
Notes on the number of non-EMV submissions to Clin Var (EMV data noted above)	Pathogenic by 4 including emv
Comment	PP5

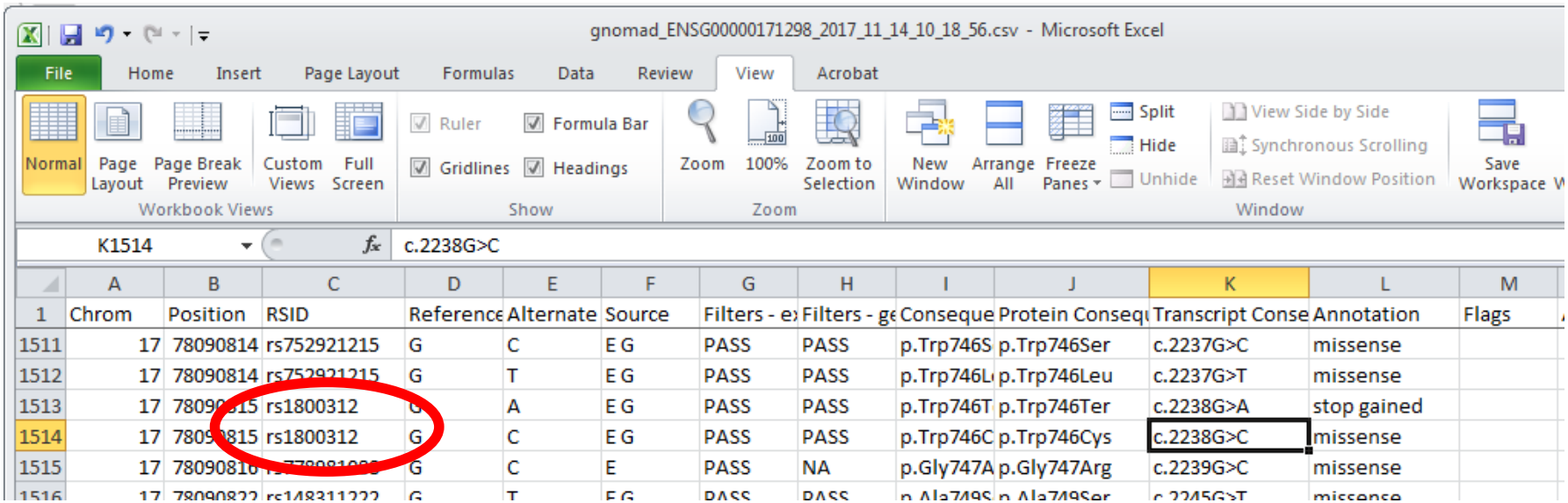
Variant Example – c.2238G>C p.(Trp746Cys)

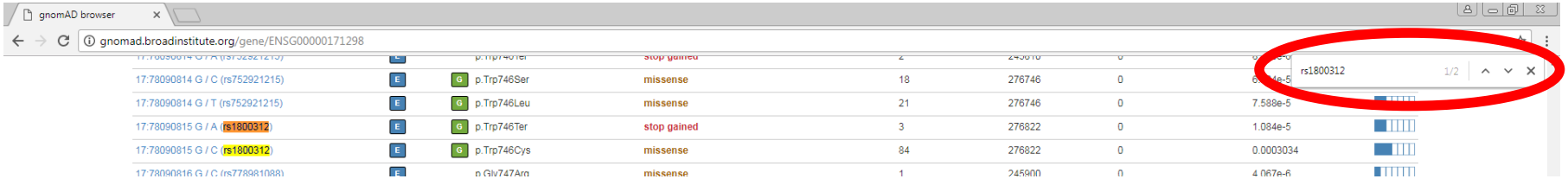
gnoMAD



Variant Example – c.2238G>C p.(Trp746Cys)

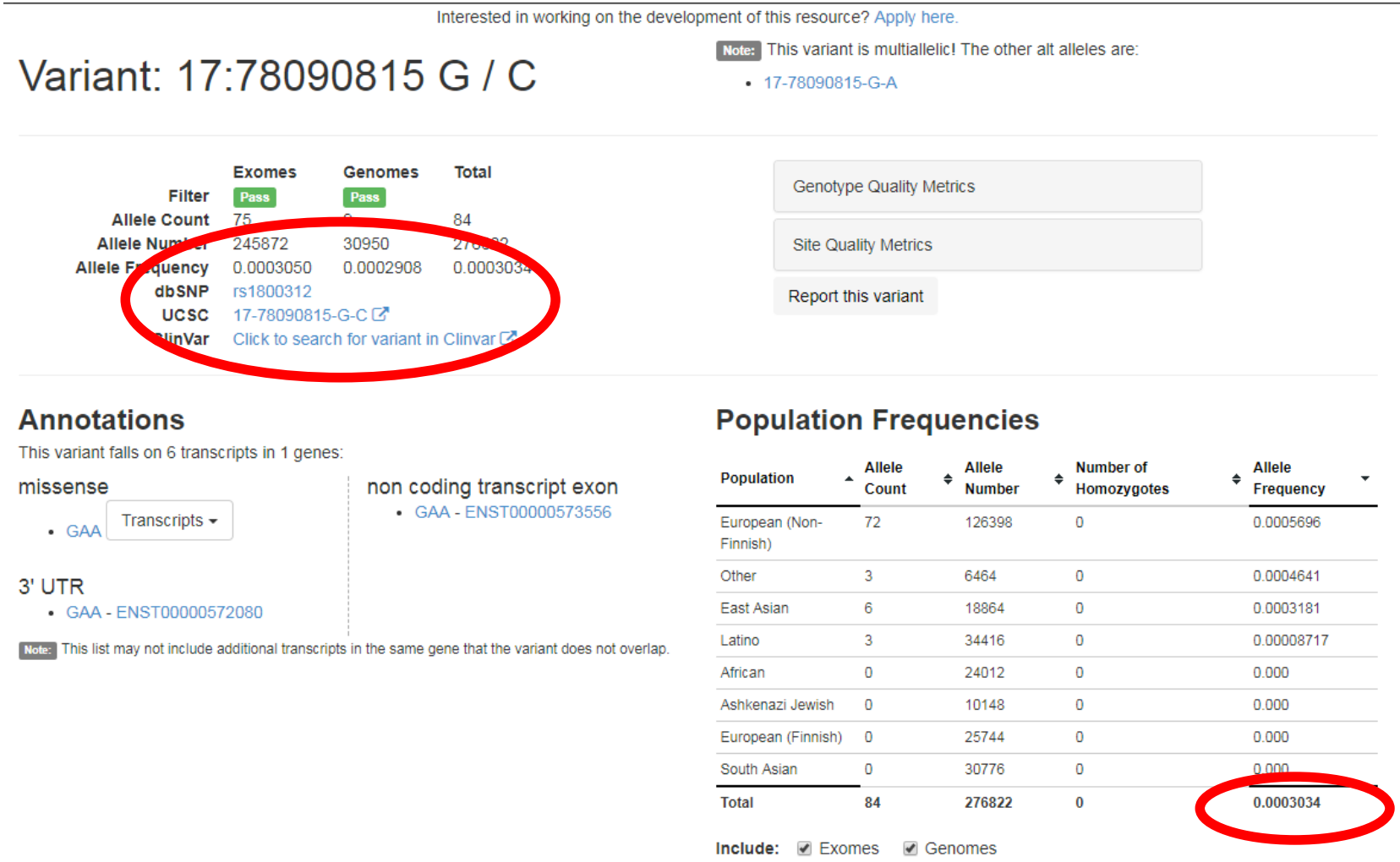
gnoMAD





Variant Example – c.2238G>C p.(Trp746Cys)

gnoMAD



Variant Example – c.2238G>C p.(Trp746Cys)

gnoMAD

VARIANT HGVS NAME: c.2238G>C p.(Trp746Cys) Date of Evaluation: 10/16/17

6. **Broad Genome Aggregation Database (gnomAD)** http://gnomad.broadinstitute.org/
   Effective use of this database requires use of the .csv file before use of the online database.
   a. Input the gene name
   b. Double click on the "Export table to CSV" - (this is an action that should be done daily, or each time a search is begun in case the database is updated).
   c. Use control F in the excel file to find your variant.
   d. Once you have found your variant, copy the rs id and use the rs id in the search of the online database (again, use the control F function). When you have found your variant in the online database, click on the rs id link and this will provide essential links to ClinVar, dbSNP, etc.

Listed?	yes
Rs id (may be in column one or csv file)	rs1800312
Consequence variant name, optional :	
Annotation:	missense
Allele freq:	0.0003034
Was there a warning about the allelic frequency – maybe insufficient data?	no
Comment:	PM2

7. **Broad Exome Aggregation Database (ExAC)** http://exac.broadinstitute.org/ SKIP THIS IF YOU GOT INFO FROM GNOMAD

Listed?	
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment	

8. **Clin Var** (*may be easily accessed through gnomad link if you found an rs id*) www.ncbi.nlm.nih.gov/clinvar/

Listed?	yes
Clinical significance	pathogenic
Last evaluated	1/25/2017
Variant Type	SNV
Rs id	
Molecular consequence	missense
Notes on the number of non-EMV submissions to Clin Var (EMV data noted above)	Pathogenic by 4 including emv
Comment	PP5

Variant Example – c.2238G>C p.(Trp746Cys)

gnoMAD

VARIANT HGVS NAME: c.2238G>C p.(Trp746Cys) Date of Evaluation: 10/16/17

9. **dbSNP** (access through gnomad link, which is best, or clin var, which means you should go through the web page's rs id to get to the best presentation of data) https://www.ncbi.nlm.nih.gov/projects/SNP/

Listed?	yes
Allele freq:	C=0.0004/2 (1000 Genomes)
Source of allelic frequency	
Comment:	

10. **UCSC genome browser** (accessed through gnomad link)

Listed?	yes
Conservation?	Amino acid conserved across all species
Comment	PP3

11. **polyphen for use with amino acid changes only** http://genetics.bwh.harvard.edu/pph2/

This mutation is predicted to be PROBABLY DAMAGING with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) PP3

12. **Other sites of desperation: google scholar etc….sites that may be of use**

PMID: 18458862 (Identification of eight novel mutations of the acid alpha-glucosidase gene causing the infantile or juvenile form of glycogen storage disease type II from J Neurol. 2008 Jun;255(6):831-8.) states

- The genotype/phenotype correlations indicated that c.2238G > C (p.W746C) is correlated with juvenile-onset GSDII
- No c.2238G > C mutations were found among the 100 normal individuals in this study. Therefore, we propose that the c.2238G > C of the GAA gene is not a polymorphism. A total of 4 out of the 12 GSDII alleles were c.2238G > C. To our knowledge, this is the first time that a c.2238G > C homozygote patient has been discovered. The homozygote noted to be 10 at age of onset and 25 at dx

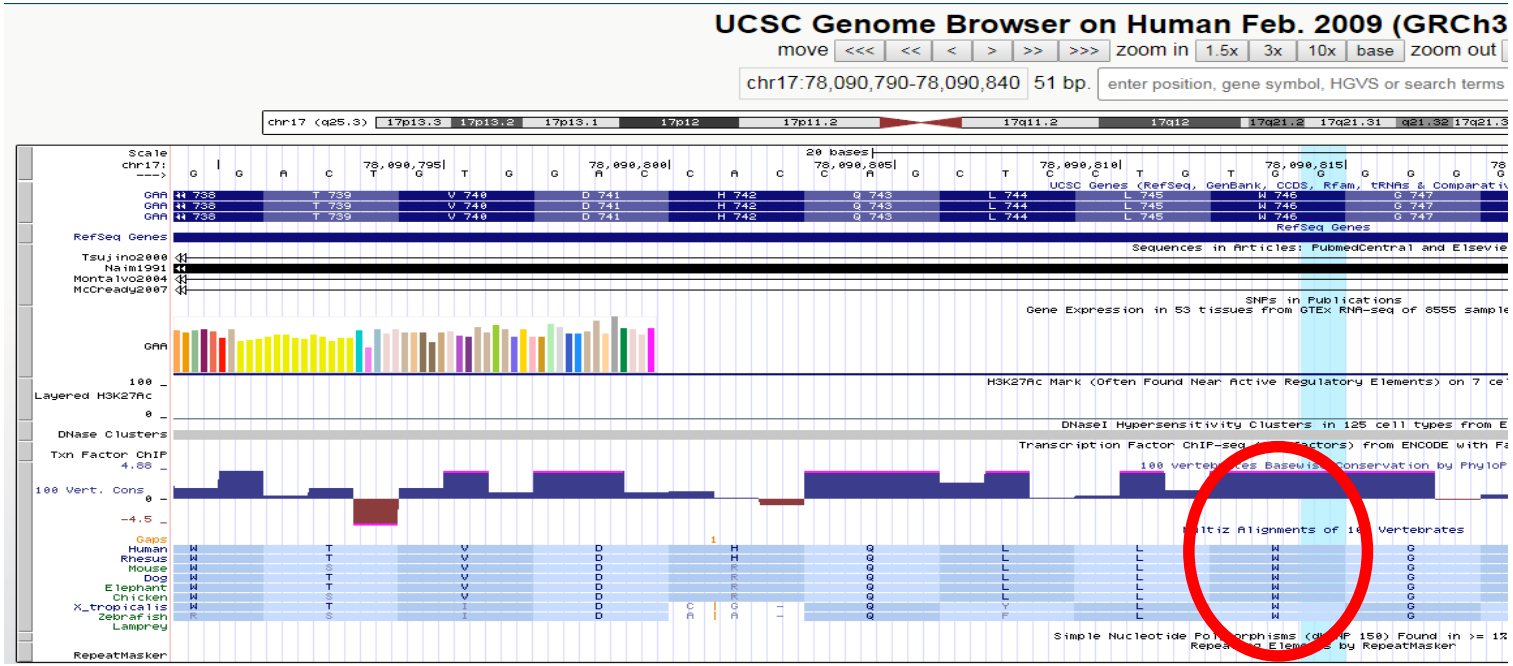
PS4 from publication

PMID: 21757382 (Rapid progressive course of later-onset Pompe disease in Chinese patients)

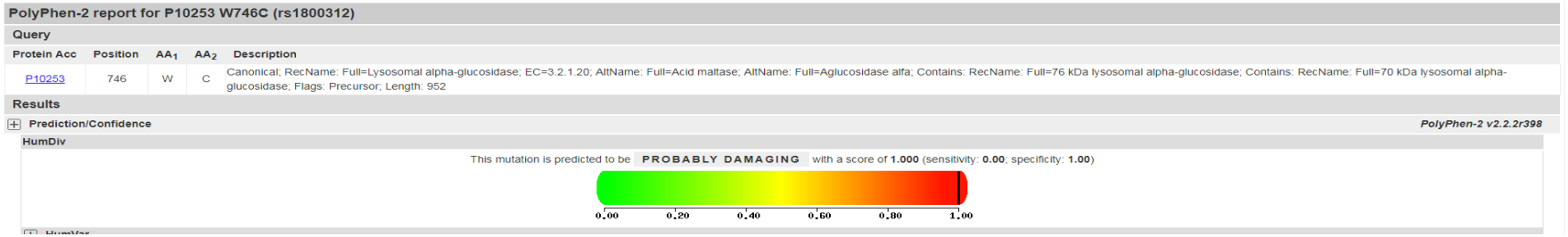
- The isolated p.W746C mutation displayed 8% of normal GAA activity in transfected fibroblast; Using mutagenesis, we showed that the p.G576S pseudodeficiency mutation significantly decreased the residual enzyme activity of p.W746C. while the p.[W746C; G576S] mutation failed to yield detectable GAA activity (0.3% of normal

PS3 from publication

UCSC Conservation Data



PolyPhen-2 Tool



Variant Example – c.2238G>C p.(Trp746Cys)

VARIANT HGVS NAME: c.2238G>C p.(Trp746Cys) Date of Evaluation: 10/16/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant** PS3, PS4, PM2, PP3, PP5 - PATHOGENIC
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	No
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

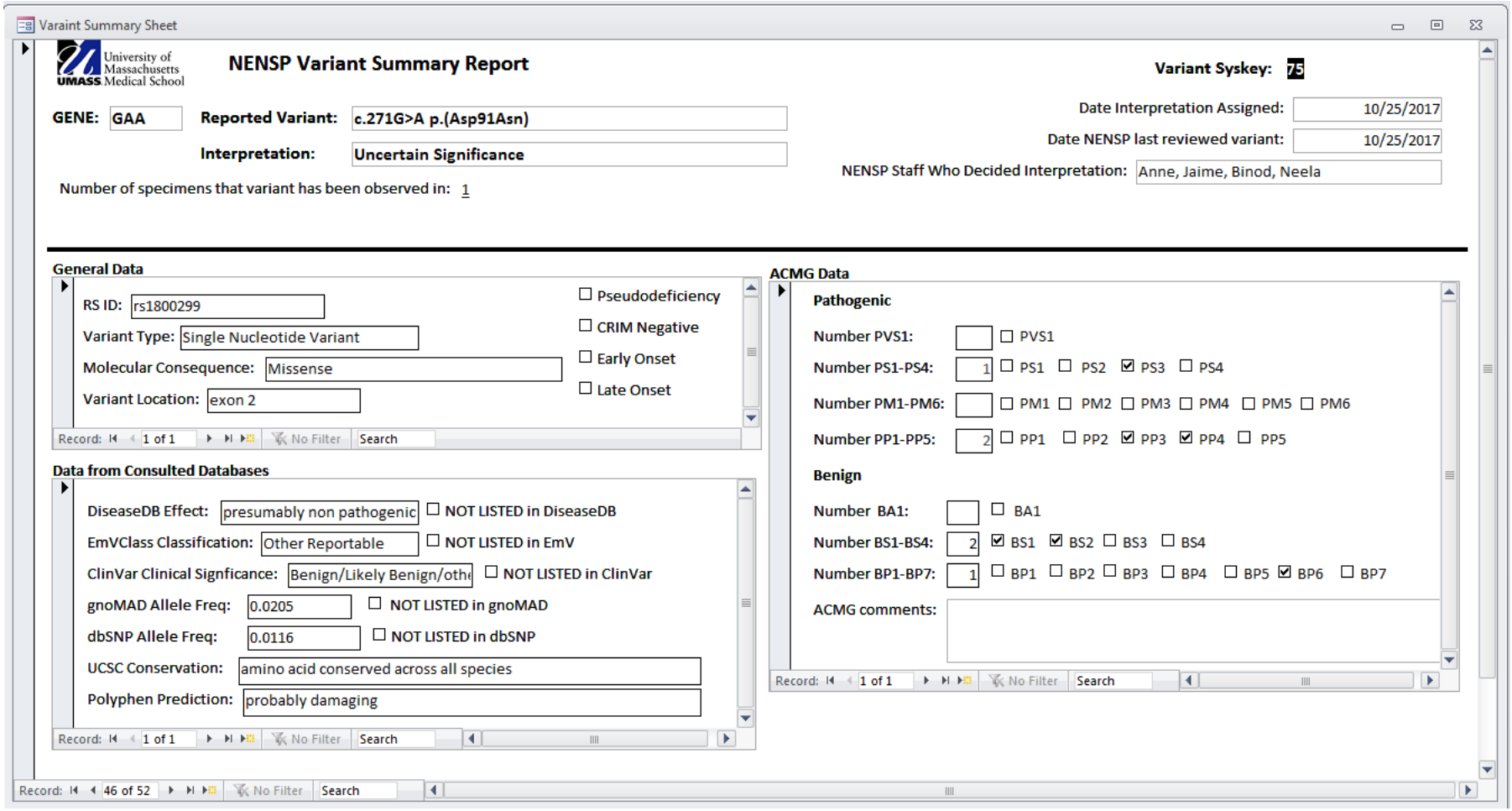
4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Exon 16
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	p.Trp746Cys
Effect:	potentially mild
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	pathogenic
Classification	
Date the variant was last reviewed.	6/15/17
Comment:	PP5

Variant Example – c.271G>A p.(Asp91Asn)



Variant Example – c.271G>A p.(Asp91Asn)

VARIANT HGVS NAME: c.271G>A p.(Asp91Asn) Date of Evaluation: 10/23/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant**
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	**none**
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) ***If you can get to the point of finding a good rs number (see below), you are in good shape.***

4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Exon 2
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	p.Asp91Asn
Effect:	presumably non-pathogenic
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	**BP6**

5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php **After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.**

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	Exon 2
Classification	Other Reportable
Date the variant was last reviewed.	11/17/16
Comment:	

Variant Example – c.271G>A p.(Asp91Asn)

VARIANT HGVS NAME: c.271G>A p.(Asp91Asn) Date of Evaluation: 10/23/17

6. **Broad Genome Aggregation Database (gnomAD)** http://gnomad.broadinstitute.org/
   Effective use of this database requires use of the .csv file before use of the online database.
   a. Input the gene name
   b. Double click on the "Export table to CSV" - (this is an action that should be done daily, or each time a search is begun in case the database is updated).
   c. Use control F in the excel file to find your variant.
   d. Once you have found your variant, copy the rs id and use the rs id in the search of the online database (again, use the control F function). When you have found your variant in the online database, click on the rs id link and this will provide essential links to ClinVar, dbSNP, etc.

Listed?	yes
Rs id (may be in column one or csv file)	rs1800299
Consequence variant name, optional :	
Annotation:	missense
Allele freq:	0.02050
Was there a warning about the allelic frequency – maybe insufficient data?	no
Comment:	BS1

7. **Broad Exome Aggregation Database (ExAC)** http://exac.broadinstitute.org/ SKIP THIS IF YOU GOT INFO FROM GNOMAD

Listed?	
Rs id (may be in column one or csv file)	
Consequence variant name, optional :	
Annotation:	
Allele freq:	
Was there a warning about the allelic frequency – maybe insufficient data?	
Comment	

8. **Clin Var** (*may be easily accessed through gnomad link if you found an rs id*) www.ncbi.nlm.nih.gov/clinvar/

Listed?	yes
Clinical significance	Benign/Likely benign, other
Last evaluated	12/8/15
Variant Type	SNV
Rs id	
Molecular consequence	missense variant
Notes on the number of non-EMV submissions to Clin Var (EMV data noted above)	Benign by 2, likely benign by 1 and other by EMV
Comment	BP6

Variant Example – c.271G>A p.(Asp91Asn)

VARIANT HGVS NAME: c.271G>A p.(Asp91Asn) Date of Evaluation: 10/23/17

9. **dbSNP** (access through gnomad link, which is best, or clin var, which means you should go through the web page's rs id to get to the best presentation of data) https://www.ncbi.nlm.nih.gov/projects/SNP/

Listed?	yes
Allele freq:	A=0.0116/58 (1000 Genomes)
Source of allelic frequency	
Comment:	

10. **UCSC genome browser** (accessed through gnomad link)

Listed?	yes
Conservation?	AA conserved across all species
Comment	PP3

11. **polyphen for use with amino acid changes only** http://genetics.bwh.harvard.edu/pph2/

This mutation is predicted to be PROBABLY DAMAGING with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) PP3

12. **Other sites of desperation: google scholar etc….sites that may be of use**

PMID: 17027861(Glycogen storage disease: clinical, biochemical, and molecular heterogeneity)

- Reports homozygote with disease PP4

PMID: 2203258 (Identification of the base-pair substitution responsible for a human acid alpha glucosidase allele with lower "affinity" for glycogen (GAA 2) and transient gene expression in deficient cells.)

- Allozyme PS3

PMID: 19387865 (Enzyme analysis for Pompe disease in leukocytes; superior results with natural substrate compared with artificial substrates.)

- calls it a pseudodeficiency

PMID: 16838077 (Seven cases of Pompe disease from Greece.)

- reported it was not known to cause disease in any combination, known as the GAA2 allele BP6

Variant Example – c.271G>A p.(Asp91Asn)

VARIANT HGVS NAME: c.271G>A p.(Asp91Asn) Date of Evaluation: 10/23/17

Variant Assessment Worksheet

1. **Condition: POMPE Variant** PS3, PP3, PP4, BS2, BP6 Conflicting evidence → Uncertain
2. **Reviewed by NENSP before?**
3. **Other newborn screening program contribution**

Name of program	**none**
Listed?	
Clinical significance	
Date last reviewed	
Comment	

Some notes about using web-based databases: Using "Control F" might save you a lot of headache and prevent the need for scrolling. Some databases are easier to use than others; some have good embedded methods for searching. Some searches seem to be particularly susceptible to spaces – e.g. in ClinVar, c.590-8 C>T might be findable and c.590-8C>T might not be – and vice versa (rules don't seem to be applied uniformly; don't give up.) *If you can get to the point of finding a good rs number (see below), you are in good shape.*

4. **Pompe database (DSdb)**
   http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html?lang=en

Date that the DATABASE was last updated:	May 2016
location	Exon 2
Hgvs DNA name in the database *optional*	
Hgvs protein name in the database *may be helpful in other searches*	p.Asp91Asn
Effect:	presumably non-pathogenic
When there are not good summary data from other sources:	
Abstracts supporting pathogenic	
Abstracts supporting vous	
Abstracts supporting benign	
Comment:	BP6

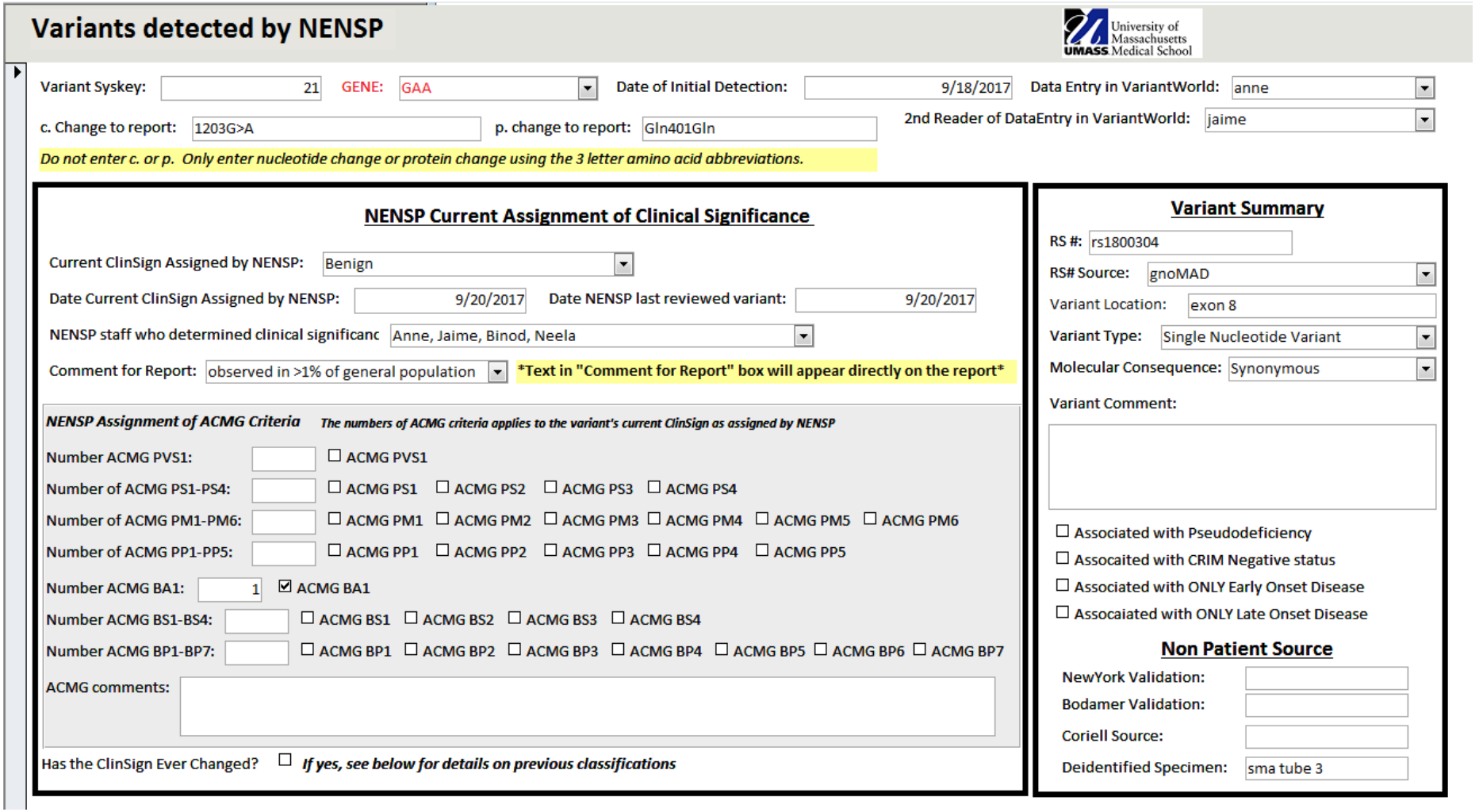
5. **EMV** www.egl-eurofins.com/emvclass/emvclass.php After inputting the gene name, in the search bar, just enter the number and list will come up. E.g., for c.973, just enter 973.

Is it listed?	yes
Location *note that intronic variants seem to be listed by closest exon*:	Exon 2
Classification	Other Reportable
Date the variant was last reviewed.	11/17/16
Comment:	

UMass Variant World – A Flat View

Variant Sysk	GENE	c Change to	p change to	Variant Type	Molecular C	RS #	Current Clin	Date Curren	DiseaseDB E	DiseaseDB c	EmVClass Cl	gnoMAD Allele Freq	Polyphen Pr	PMID1
64	GAA	2189+95C>T					Uncertain Signi	10/16/2017		☑				
65	GAA	858+5_858+6in:					Uncertain Signi	10/20/2017		☑	Benign			
66	GAA	858+6_858+7in:								☐				
67	GAA	858+7_858+8 in								☐				
68	GAA	2238G>C	Trp746Cys	Single Nucleot	Missense	rs1800312	Pathogenic	10/20/2017	potentially mil	☐	Pathogenic	0.00003034	probably dama	18458862
70	GAA	2331+84C>G					Uncertain Signi	10/20/2017		☑				
71	GAA	2444A>G	Asn815Ser		Missense		Uncertain Signi	10/20/2017		☑		0.00001241	benign	
72	IDUA	942G>C	Ala314Ala	Single Nucleot	Synonymous	rs6830825	Benign	10/4/2017		☑	Benign			
73	IDUA	1230C>G	Thr410Thr	Single Nucleot	Synonymous	rs115790973	Benign	9/29/2017		☑	Benign	0.1717		
74	IDUA	1360G>A	Val454Ile	Single Nucleot	Missense	rs73066479	Benign	9/29/2017		☑	Benign	0.1862		
75	GAA	271G>A	Asp91Asn	Single Nucleot	Missense	rs1800299	Uncertain Signi	10/25/2017	presumably no	☐	Other Reportal	0.0205	probably dama	17027861
76	GAA	307T>G	Cys103Gly	Single Nucleot	Missense	rs398123174	Pathogenic	10/25/2017	potentially les	☐	Pathogenic	0.00003233	probably dama	14695532
77	GAA	1000G>T					Uncertain Signi	10/25/2017		☐				
78	ABCD1	1992-32C>T		Single Nucleot	Intron Variant	rs4898368	Benign	11/1/2017		☑	Benign	0.6239		
79	ABCD1	901-70A>G					Uncertain Signi	11/1/2017		☑				
80	ABCD1	1390C>T	Arg464Ter	Single Nucleot	Nonsense	rs128624221	Pathogenic	11/1/2017	pathogenic	☐	Pathogenic			21700483
81	ABCD1	2238+8G>C			3 prime UTR va		Benign	11/1/2017	benign based c	☐		0.6782		
82	IDUA	-409A>G				rs4690220	Benign	11/20/2017		☑				
83	IDUA	-272C>G				rs189844468	Uncertain Signi	11/20/2017		☑				
84	IDUA	24C>A	Ala8Ala	Single Nucleot	Synonymous	rs11248061	Benign	11/20/2017		☑	Benign			
85	IDUA	60G>A	Ala20Ala	Single Nucleot	Synonymous	rs10902762	Benign	11/20/2017		☑	Benign			
86	IDUA	208C>T	Gln70Ter	Single Nucleot	Nonsense	rs121965020	Pathogenic	11/20/2017		☑	Pathogenic	0.0005257		12865757
87	IDUA	300-44C>T		Single Nucleot	Intron Variant	rs3755956	Likely Benign	11/20/2017		☑	Benign	0.03679		
88	IDUA	709C>T	Leu237Phe	Single Nucleot	Missense	rs74385837	Uncertain Signi	11/20/2017		☑		0.001617	probably dama	
89	IDUA	793-1G>A		Single Nucleot	Splice Accepto	rs762779421	Uncertain Signi	11/20/2017		☑		0.00001836		
90	IDUA	891C>T	Asn297Asn	Single Nucleot	Synonymous	rs114806891	Benign	11/20/2017		☑	Benign	0.05348		21394825
91	IDUA	1029C>A	Tyr343Ter	Single Nucleot	Nonsense	rs764196171	Likely Pathoge	11/20/2017		☑				9391892
92	IDUA	1205G>A	Trp402Ter	Single Nucleot	Nonsense	rs121965019	Pathogenic	11/20/2017		☑	Pathogenic	0.0006687		11735025
93	IDUA	1224C>T	Ala408Ala	Single Nucleot	Synonymous	rs774488302	Uncertain Signi	11/29/2017		☑		0.00003419		
94	IDUA	1402+1G>C		Single Nucleot	Splice Donor V	res398123254	Pathogenic	11/29/2017		☑	Pathogenic			
95	IDUA	1469T>C	Leu490Pro	Single Nucleot	Missense	rs121965027	Likely Pathoge		attenuated-int	☐	Pathogenic		benign	7550232
96	GAA	921A>T	Ala307Ala	Single Nucleot	Synonymous	rs1800303	Benign	11/29/2017	non-pathogeni	☐	Benign	0.07629		
97	GAA	1374C>T	Tyr458Tyr	Single Nucleot	Synonymous	rs1800305	Benign	11/29/2017	non-pathogeni	☐	Benign	0.07257		
98	GAA	2237G>T	Trp746Leu	Single Nucleot	Missense	rs752921215	Uncertain Signi			☑	VOUS	0.00007588	probably dama	
99	GAA	878G>A	Gly293Glu				Uncertain Signi	11/29/2017		☑			probably dama	
100	GAA	2069C>t	Pro690Leu	Single Nucleot	Missense	rs532624326	Uncertain Signi	12/4/2017		☑		0.00006467	benign	
101	GAA	-32-13T>G		Single Nucleot	Intron Variant	rs386834236	Pathogenic	12/4/2017	POTENTIALLY M	☐	Pathogenic	0.003414		22676651
102	GAA	1888+5G>T		Single Nucleot	Intron Variant/	rs528282884	Uncertain Signi	12/4/2017		☑	VOUS	0.00002378		
103	GAA	2297A>C	Tyr766Serr	Single Nucleot	Missense	rs144016984	Likely Pathoge	1/31/2018		☑		0.000004076	probably dama	26693141

Variant Entry Form Part 1



Variants detected by NENSP

University of Massachusetts Medical School

Variant Syskey: 21 GENE: GAA Date of Initial Detection: 9/18/2017 Data Entry in VariantWorld: anne

c. Change to report: 1203G>A p. change to report: Gln401Gln 2nd Reader of DataEntry in VariantWorld: jaime

Do not enter c. or p. Only enter nucleotide change or protein change using the 3 letter amino acid abbreviations.

NENSP Current Assignment of Clinical Significance

Current ClinSign Assigned by NENSP: Benign

Date Current ClinSign Assigned by NENSP: 9/20/2017 Date NENSP last reviewed variant: 9/20/2017

NENSP staff who determined clinical significanc: Anne, Jaime, Binod, Neela

Comment for Report: observed in >1% of general population *Text in "Comment for Report" box will appear directly on the report*

NENSP Assignment of ACMG Criteria *The numbers of ACMG criteria applies to the variant's current ClinSign as assigned by NENSP*

Number ACMG PVS1: ☐ ACMG PVS1

Number of ACMG PS1-PS4: ☐ ACMG PS1 ☐ ACMG PS2 ☐ ACMG PS3 ☐ ACMG PS4

Number of ACMG PM1-PM6: ☐ ACMG PM1 ☐ ACMG PM2 ☐ ACMG PM3 ☐ ACMG PM4 ☐ ACMG PM5 ☐ ACMG PM6

Number of ACMG PP1-PP5: ☐ ACMG PP1 ☐ ACMG PP2 ☐ ACMG PP3 ☐ ACMG PP4 ☐ ACMG PP5

Number ACMG BA1: 1 ☑ ACMG BA1

Number ACMG BS1-BS4: ☐ ACMG BS1 ☐ ACMG BS2 ☐ ACMG BS3 ☐ ACMG BS4

Number ACMG BP1-BP7: ☐ ACMG BP1 ☐ ACMG BP2 ☐ ACMG BP3 ☐ ACMG BP4 ☐ ACMG BP5 ☐ ACMG BP6 ☐ ACMG BP7

ACMG comments:

Has the ClinSign Ever Changed? ☐ *If yes, see below for details on previous classifications*

Variant Summary

RS #: rs1800304

RS# Source: gnoMAD

Variant Location: exon 8

Variant Type: Single Nucleotide Variant

Molecular Consequence: Synonymous

Variant Comment:

☐ Associated with Pseudodeficiency

☐ Assocaited with CRIM Negative status

☐ Associated with ONLY Early Onset Disease

☐ Assocaiated with ONLY Late Onset Disease

Non Patient Source

NewYork Validation:

Bodamer Validation:

Coriell Source:

Deidentified Specimen: sma tube 3

Variant Entry Form Part 2

Data from consulted databases ☐ Reported ClinSign varies between DBs

DiseaseDB | EmVClass | gnoMAD | ExAC | ClinVar | dbSNP | UCSC | Polyphen

DiseaseDB date last updated: 5/31/2016

☐ DiseaseDB checked NOT LISTED

DiseaseDB Effect: very severe

DiseaseDB date last consulted by NENSP: 10/4/2017

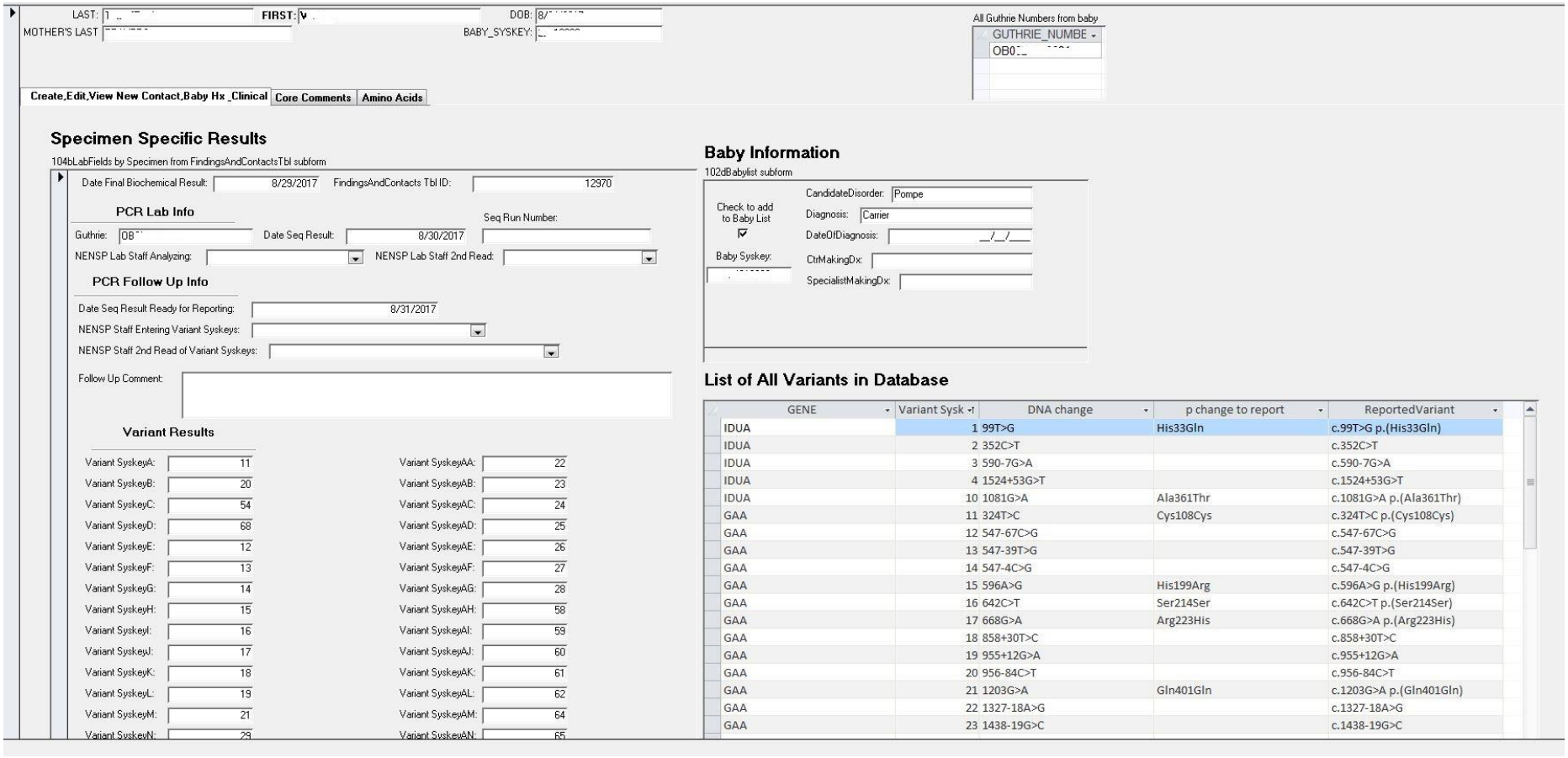
The field DiseaseDB Effect is listed in the Disease DBs as follows:
Pompe DB: Effect Pompe DiseaseDB
ALD DB: Remark ALD DiseaseDB
MPS1: ptype MPS1 DiseaseDB

DiseaseDB Comment: publications suggest late onset see below

PMID Citations consulted Link to PubMed

PMID1:	7881425	PMID3:	25673129	PMID5:	
PMID1 comment:	adult onset	PMID3 comment:	reported as very severe in six patients with adult onset	PMID5 comment:	
PMID2:	11343339	PMID4:	17056254	PMID6:	
PMID2 comment:	one patitent with infantile onset a related publication with this infant had 40 something adult onset patients	PMID4 comment:	alters conserved splice donor site in intron ten; disrupts splicing leads to inframe splicing intron 10 functionally null	PMID6 Comment:	

Specimen Variant Entry Form



Report to Healthcare Provider

University of Massachusetts Medical School
UMASS Medical School

New England Newborn Screening Program
University of Massachusetts Medical School
Biotech 4, 2nd Floor
377 Plantation Street
Worcester, MA 01605-2300
774-455-4600 (phone) 774-455-4657 (fax)

Report Attachment of Sequencing Results for Gene Associated with Pompe Disease

Report Date: 11/10/2017 Lab Number: [redacted]

Name of Baby: [redacted]

Date of Birth: [redacted]/2017 5:09:00 AM

Name of Mother: [redacted]

Name of Gene Sequenced: GAA

Name of Variant(s) Detected	Interpretation of Variant(s)	Comment
c.1551+1G>C p.(Val480_Ile517del)	Pathogenic	
c.2189+95C>T	Uncertain Significance	
c.99T>G p.(His33Gln)	Benign	observed in >1% of general population

Some variants in the acid α-glucosidase (GAA) gene are associated with low levels of enzyme activity. Low enzyme activity can result in the accumulation of lysosomal glycogen and cause Pompe Disease. The condition has a broad phenotype, ranging from an infantile form associated with significant morbidity and death in early childhood to a late-onset form, associated with progressive weakness and respiratory failure, with highly variable onset and progression. Pompe Disease is an autosomal recessive disease.

Note: The variants c.1726G>A p.(Gly576Ser) and c.2065G>A p.(Glu689Lys) also called c.[1726A; 2065A] are known to be associated with a pseudodeficiency of acid α-glucosidase (1).

Note: The variants c.2560C>T p.(Arg854Ter) and c.525delT p.(Glu176ArgfsTer45) are the two most common variants known to be associated with Cross Reactive Immunological Material (CRIM) negative status; however, many other variants are also associated with CRIM negative status (2).

Sequencing results were generated by fluorescence based Sanger sequence analysis of specific exonic and intronic regions of the GAA gene. This test will not detect all large deletions, duplications, or deep intronic variants. Phasing of detected variants cannot be predicted without parental testing.

(1) The Condition Review Workgroup for the Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children. Evidence Report: Newborn Screening for Pompe Disease. 2013. (https://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/pompereport2013.pdf)

(2) Bali D, Goldstein J, Banugaria S, et al. Predicting cross reactive immunological material (CRIM) status in pompe disease using GAA mutations: Lessons learned from 10 years of clinical laboratory testing experience. Molecular Genetics and Metabolism. 2012;105(2):520.

This test has not been cleared or approved by the FDA. However, the New England Newborn Screening Program determined the performance characteristics of the test and the FDA has determined that its clearance and approval are not required for NENSP-specific uses.

Test performed by New England Newborn Screening Program, 377 Plantation St, Worcester, MA 01605 Roger B. Eaton, Ph.D., Director

Please contact the New England Newborn Screening Program at 774-455-4600 if you have any quetions regarding this report.

Report to Healthcare Provider

University of Massachusetts Medical School
UMASS

New England Newborn Screening Program
University of Massachusetts Medical School
Biotech 4, 2nd Floor
377 Plantation Street
Worcester, MA 01605-2300
774-455-4600 (phone) 774-455-4657 (fax)

Report Attachment of Sequencing Results for Gene Associated with Pompe Disease

Report Date: 2/13/2018 Lab Number: [redacted]

Name of Baby: [redacted],

Date of Birth: [redacted] PM

Name of Mother: [redacted],

Name of Gene Sequenced: GAA

Name of Variant(s) Detected	Interpretation of Variant(s)	Comment
c.2238G>C p.(Trp746Cys)	Pathogenic	
c.2238G>C p.(Trp746Cys)	Pathogenic	
c.1581G>A p.(Arg527Arg)	Benign	observed in >1% of general population

The GAA gene and Pompe Disease (additional information included on all reports)

Some variants in the acid α-glucosidase (GAA) gene are associated with low levels of enzyme activity. Low enzyme activity can result in the accumulation of lysosomal glycogen and cause Pompe Disease. The condition has a broad phenotype, ranging from an infantile form associated with significant morbidity and death in early childhood to a late-onset form, associated with progressive weakness and respiratory failure, with highly variable onset and progression. Pompe Disease is an autosomal recessive disease.

Note: The variants c.1726G>A p.(Gly576Ser) and c.2065G>A p.(Glu689Lys) also called c.[1726A; 2065A] are known to be associated with a pseudodeficiency of acid α-glucosidase (1).

Note: The variants c.2560C>T p.(Arg854Ter) and c.525delT p.(Glu176ArgfsTer45) are the two most common variants known to be associated with Cross Reactive Immunological Material (CRIM) negative status; however, many other variants are also associated with CRIM negative status (2).

Sequencing results were generated by fluorescence based Sanger sequence analysis of specific exonic and intronic regions of the GAA gene. This test will not detect all large deletions, duplications, or deep intronic variants. Phasing of detected variants cannot be predicted without parental testing.

(1) The Condition Review Workgroup for the Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children. Evidence Report: Newborn Screening for Pompe Disease. 2013. (https://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/pompereport2013.pdf)

(2) Bali D, Goldstein J, Banugaria S, et al. Predicting cross reactive immunological material (CRIM) status in pompe disease using GAA mutations: Lessons learned from 10 years of clinical laboratory testing experience. Molecular Genetics and Metabolism. 2012;105(2):320.

This test has not been cleared or approved by the FDA. However, the New England Newborn Screening Program determined the performance characteristics of the test and the FDA has determined that its clearance and approval are not required for NENSP-specific uses.

Test performed by New England Newborn Screening Program, 377 Plantation St, Worcester, MA 01605 Roger B. Eaton, Ph.D., Director

Report to Healthcare Provider

University of Massachusetts Medical School

New England Newborn Screening Program
University of Massachusetts Medical School
Biotech 4, 2nd Floor
377 Plantation Street
Worcester, MA 01605-2300
774-455-4600 (phone) 774-455-4657 (fax)

Report Attachment of Sequencing Results for Gene Associated with MPS1

Report Date: 2/13/2018 Lab Number: [redacted]

Name of Baby: [redacted],

Date of Birth: [redacted] AM

Name of Mother: [redacted], [redacted]

Name of Gene Sequenced: IDUA

Name of Variant(s) Detected	Interpretation of Variant(s)	Comment
c.590-7G>A	Likely Pathogenic	
c.300-44C>T	Likely Benign	observed in >1% of general population

The IDUA gene and MPS1 (additional information included on all reports)

Some variants in the α-L-iduronidase (IDUA) gene are associated with low levels of enzyme activity. Low enzyme activity can result in the accumulation of glycosaminoglycans and cause mucopolysaccharidosis type I (MPS1). The condition is a progressive multisystem disorder with a broad spectrum of disease onset and severity. MPS1 is sometimes classified as one of three types– Hurler syndrome, Hurler-Scheie syndrome, or Scheie syndrome and somtimes described as severe or attenuated depending on age of onset. Severe MPS1 usually presents during the first or second year of life, with pervasive, multi-systemic involvement and rapid disease progression leading to death by 10 years of age. The attenuated form can onset around age three years through 12 years, though may also occur later in adulthood and typically progresses more slowly than the severe form. MPS1 is an autosomal recessive disease.

Note: The variants c.235G>A (p.Ala79Thr), c.246C>G (p.His82Gln), c.667G>A (p.Asp223Asn) and c.965T>A (p.Val322Glu) are known to be associated with a pseudodeficiency of α-L-iduronidase (1).

Sequencing results were generated by fluorescence based Sanger sequence analysis of specific exonic and intronic regions of the IDUA gene. This test will not detect all large deletions, duplications, or deep intronic variants. Phasing of detected variants cannot be predicted without parental testing.

(1). Pollard L, Braddoc S, Christensen K, Boylan D, Heese B. Diagnostic follow-up of 47 infants with a positive newborn screen for Hurler syndrome: identification of four recurrent IDUA sequence changes that significantly reduce enzyme activity. In: APHL meeting, Anaheim, CA. Proceedings of the 2014 APHI newborn screening and genetic testing symposium, Anaheim, CA, October 27-30, 2014. (https://www.aphl.org/conferences/Documents/Follow-up-2.pdf)

This test has not been cleared or approved by the FDA. However, the New England Newborn Screening Program determined the performance characteristics of the test and the FDA has determined that its clearance and approval are not required for NENSP-specific uses.

Test performed by New England Newborn Screening Program, 377 Plantation St, Worcester, MA 01605 Roger B. Eaton, Ph.D., Director

Please contact the New England Newborn Screening Program at 774-455-4600 if you have any quetions regarding this report.

Report to Healthcare Provider

University of Massachusetts Medical School
UMASS

New England Newborn Screening Program
University of Massachusetts Medical School
Biotech 4, 2nd Floor
377 Plantation Street
Worcester, MA 01605-2300
774-455-4600 (phone) 774-455-4657 (fax)

Report Attachment of Sequencing Results for Gene Associated with X-ALD

Report Date: 2/13/2018 Lab Number: [redacted]

Name of Baby: [redacted], [redacted]

Date of Birth: [redacted] PM

Name of Mother: [redacted], [redacted]

Name of Gene Sequenced: ABCD1

Name of Variant(s) Detected	Interpretation of Variant(s)	Comment
c.1390C>T p.(Arg464Ter)	Pathogenic	
c.901-70A>G	Uncertain Significance	

The ABCD1 gene and X-ALD (additional information included on all reports)

The ATP binding cassette subfamily D member 1 (ABCD1) gene encodes a protein called adrenoleukodystrophy protein (ADLP) and some variants in this gene lead to a lack of or defective ADLP. Absence or dysfunctional ADLP can lead to an accumulation of very long chain fatty acids that damages the adrenal glands, the brain, and the spinal cord and causes adrenoleukodystrophy (ALD). Due to an X-linked inheritance pattern, the most severe, childhood cerebral form onsets in boys between the ages of 4 and 10, beginning with behavioral issues and rapidly progresses to a vegetative state with blindness, deafness, seizures, loss of muscle control, and progressive dementia. Adrenomyeloneuropathy or Addison disease can develop later in life. Many female carriers develop clincal signs of ALD in late adulthood.

Sequencing results were generated by fluorescence based Sanger sequence analysis of specific exonic and intronic regions of the ABCD1 gene. This test will not detect all large deletions, duplications, or deep intronic variants. Phasing of detected variants cannot be predicted without parental testing.

This test has not been cleared or approved by the FDA. However, the New England Newborn Screening Program determined the performance characteristics of the test and the FDA has determined that its clearance and approval are not required for NENSP-specific uses.

Test performed by New England Newborn Screening Program, 377 Plantation St, Worcester, MA 01605 Roger B. Eaton, Ph.D., Director

Please contact the New England Newborn Screening Program at 774-455-4600 if you have any quetions regarding this report.

Contact

Anne Comeau, PhD
New England Newborn Screening Program
anne.comeau@umassmed.edu
774-455-4600

Commonwealth Medicine
University of Massachusetts Medical School
333 South Street, Shrewsbury MA 01545