Genomics & Proteomics & Significance Tests

Question 1

Firstly, the single nucleotide variations (SNVs) present in the table (Table 1.) have been obtained from the gnomAD database. The information pertaining to the SNVs is reported below in sequence.

Table 1.

4-105236371-A-G
17-746331-T-C
4-1395373-A-G
19-48628944-T-C

Variant: 4-105236371-A-G

Link: https://gnomad.broadinstitute.org/variant/4-105236371-A-G?dataset=gnomad r4

a) Total population frequency is 0.0006202 for the SNV. The lowest frequency is 0.00004392 for South Asians. The highest frquency is 0.007793 for African/African American. We can check the all population frequency, count and number of homozygotes informations using the table in Figure 1.

gnomAD HGDP 1KG Local Ance	estry			
Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African/African American	585	75066	3	0.007793
Admixed American	106	59998	1	0.001767
Remaining	62	62510	1	0.0009918
Middle Eastern	6	6062	0	0.0009898
European (non-Finnish)	233	1180006	1	0.0001975
East Asian	5	44862	0	0.0001115
South Asian	4	91082	0	0.00004392
European (Finnish)	0	63928	0	0.000
Ashkenazi Jewish	0	29602	0	0.000
Amish	0	912	0	0.000
XX	531	812402	1	0.0006536
XY	470	801626	5	0.0005863

Figure 1. Population Frequency Table for 4-105236371-A-G.

Gene position is 4-105145875-105279816 (region) for 4-105236371-A-G. (https://gnomad.broadinstitute.org/region/4-105145875-105279816?dataset=gnomad_r4)
The gene position is displayed in Figure 2. The variant in question is observed in 6 transcripts of the *tet methylcytosine dioxygenase (TET2)* gene.

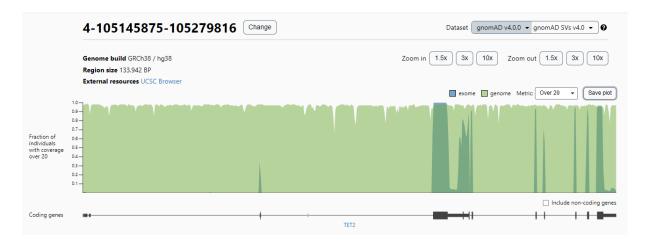


Figure 2. 4-105236371-A-G's gene position.

b) REVEL and PolyPhen Scores

While **REVEL** score is **0.0780**, **Polyphen** (max) score is **0.0100**. The REVEL score of 0.0780 generally indicates a lower probability of pathogenicity. The PolyPhen score, which attempts to predict the effects of a variant on the function of a protein, is 0.0100, typically suggesting a lower probability of pathogenicity as well. Both scores ultimately demonstrate a lower pathogenicity characteristic. The low PolyPhen score can be "non-synonymous".



Figure 3. In Silico Predictors.

c) ClinVar Informations

According to ClinVar data, the variant displaying uncertain significance and benign/likely benign characteristics is assessed to have a lower tendency for disease manifestation. These findings from ClinVar are consistent with our REVEL and PolyPhen scores.

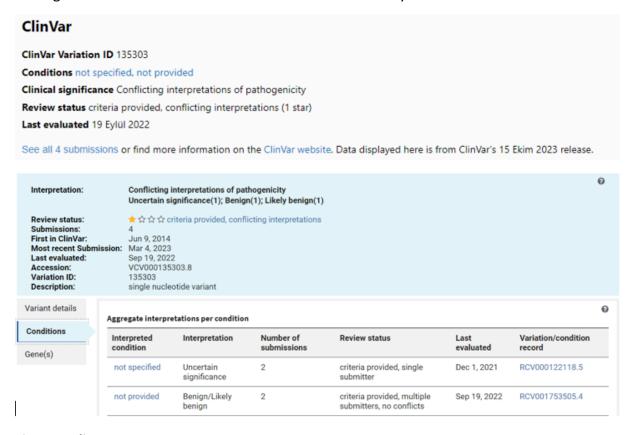


Figure 4. ClinVar Report.

d) Variant Filter/Prioritize

The variant in question exhibits missense characteristics; however, based on the scores and information obtained from gnomAD and ClinVar, the likelihood of it being pathogenic is low. In addition to there are conflicting comments regarding pathogenicity.

Variant: 17-746331-T-C

Link: https://gnomad.broadinstitute.org/variant/17-746331-T-C?dataset=gnomad r4

a) Total population frequency is **0.005788** for the SNV. The lowest frequency is 0.0003565 for East Asians. The highest frequency is 0.01171for Middle Eastern. We can check the all population frequency, count and number of homozygotes informations using the table in Figure 5.

gnomAD HGDP 1KG Local Ance	estry			
Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Middle Eastern	71	6062	1	0.01171
• Amish	8	912	0	0.008772
South Asian	622	91086	5	0.006829
Remaining	416	62484	0	0.006658
European (non-Finnish)	7683	1179872	30	0.006512
Admixed American	248	60020	0	0.004132
Ashkenazi Jewish	59	29608	0	0.001993
African/African American	143	75008	1	0.001906
European (Finnish)	74	63818	0	0.001160
East Asian	16	44880	0	0.0003565
XX	4649	812292	17	0.005723
XY	4691	801458	20	0.005853

Figure 5. Population Frequency Table for 17-746331-T-C

Gene position is 17-744421-753999 for **17-746331-T-C**; displayed the Figure 7. The variant in question is observed in 6 transcripts of the *gem nuclear organelle associated protein 4 (GEMIN4)* gene.

https://gnomad.broadinstitute.org/region/17-744421-753999?dataset=gnomad r4

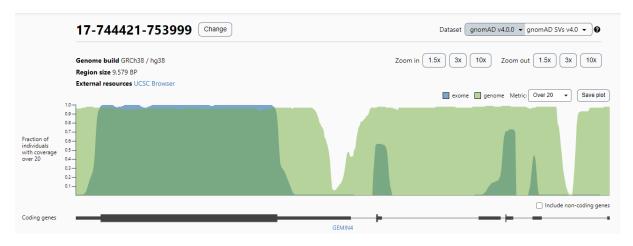


Figure 6. Gene Position for 17-746331-T-C

b) REVEL and PolyPhen Scores

While **REVEL** score is **0.295**, **Polyphen (max)** score is **0.996**. The REVEL score of 0.295 for the genetic variant indicates a low likelihood of a pathogenic effect, while the PolyPhen (max) score of 0.996 is considerably high, suggesting the potential for a pathogenic impact. However, it is important to note that although a high PolyPhen score implies the potential harmfulness of the genetic variant, in specific contexts and diseases, there is also a possibility that no harmful effect may be observed.

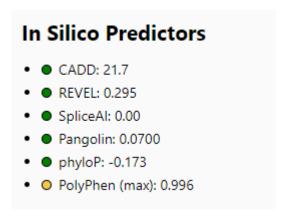


Figure 7. In Silico Predictors.

c) ClinVar Informations

According to data obtained from ClinVar, the variant exhibits characteristics indicative of being likely benign and does not display any features of pathogenicity. However, the notable high PolyPhen score of 0.996 is a point of attention. The elevated PolyPhen score of 0.996 suggests a high likelihood of pathogenicity.

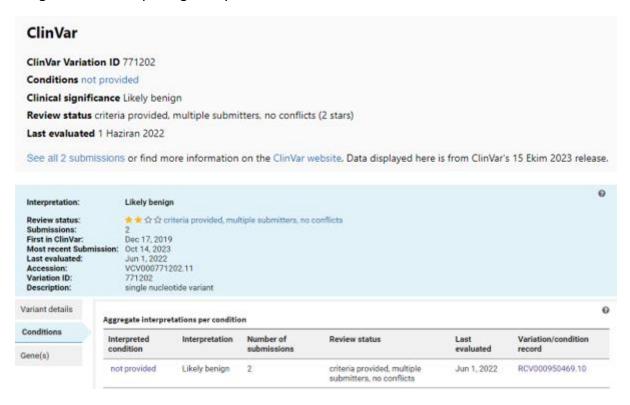


Figure 8. ClinVar Report.

d) Variant Filter/Prioritize

The variant is characterized as missense, and studies conducted have not indicated any harmful effects. However, this does not necessarily imply the absence of direct pathogenicity.

Variant: 4-1395373-A-G

Link: https://gnomad.broadinstitute.org/variant/4-1395373-A-G?dataset=gnomad r4

a) Total population frequency is **0.3576** for the SNV. The lowest frequency is 0.05896 for East Asians. The highest frquency is 0.3944 for European(non-Finnish). We can check the all population frequency, count and number of homozygotes informations using the table in Figure 9.

gnomAD HGDP 1KG Local Ancestry						
Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency	•	
European (non-Finnish)	411130	1042334	91674	0.3944		
European (Finnish)	18017	45780	4329	0.3936		
Remaining	18322	54598	3654	0.3356		
Ashkenazi Jewish	7922	24574	1546	0.3224		
Admixed American	13466	45772	2527	0.2942		
South Asian	20648	84166	2997	0.2453		
Middle Eastern	1060	4732	176	0.2240		
Amish	33	238	11	0.1387		
African/African American	4565	49442	497	0.09233		
East Asian	2320	39346	98	0.05896		
XX	251476	697746	54789	0.3604		
XY	246007	693236	52720	0.3549		
Total	497483	1390982	107509	0.3576		

Figure 9. Population Frequency for 4-1395373-A-G

Gene position is 4-1345691-1395989 for **4-1395373-A-G**; displayed the gene position on Figure 10. The variant in question is observed in 6 transcripts of the *UV stimulated scaffold protein A*

(UVSSA) gene.

https://gnomad.broadinstitute.org/gene/ENSG00000163945?dataset=gnomad r4

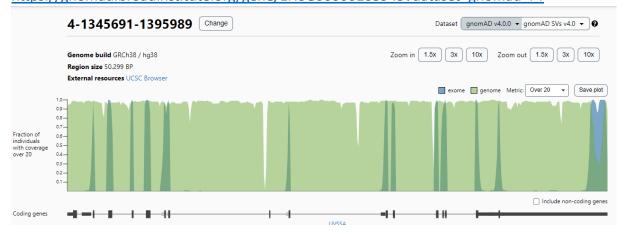


Figure 10. Gene Position for 4-1395373-A-G

b) REVEL and PolyPhen Scores

The variant hasn't got REVEL and PolyPhen scores; however it has got CADD, SpliceAl, Pangolin, and phyloP scores.

In Silico Predictors

CADD: 6.27

SpliceAl: 0.00

Pangolin: 0.00

phyloP: 0.133

Figure 11. In Silico Predictors.

c) ClinVar Informations

Not reported in ClinVar.

d) Variant Filter/Prioritize

The ClinVar data, as well as REVEL and PolyPhen scores specific to the relevant variant, are not available. Therefore, we cannot make a direct inference about the likelihood of the variant causing the disease and its pathogenicity.

Variant: 19-48628944-T-C

Link: https://gnomad.broadinstitute.org/variant/19-48628944-T-C?dataset=gnomad r4

Total population frequency is **0.00003204** for the SNV. The lowest frequency is 0.000008608 for European(non-Finnish). The highest frquency is 0.001795 for Middle Eastern. We can check the all population frequency, count and number of homozygotes informations using the table in Figure 1.

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency	•
Middle Eastern	7	3900	0	0.001795	
Remaining	3	32902	0	0.00009118	
African/African American	1	17572	0	0.00005691	
East Asian	2	35784	0	0.00005589	
South Asian	3	69598	0	0.00004310	
Admixed American	1	43342	0	0.00002307	
European (non-Finnish)	3	348500	0	0.00008608	
European (Finnish)	0	51760	0	0.000	
Ashkenazi Jewish	0	20890	0	0.000	
XX	5	284162	0	0.00001760	
XY	15	340086	0	0.00004411	
Total	20	624248	0	0.00003204	

Figure 12. Population Frequency for 19-48628944-T-C.

Gene position is 19-48619291-48630717 for **19-48628944-T-C**; displayed the gene position on Figure 10. The variant in question is observed in 6 transcripts of the *sphingosine kinase 2* (*SPHK2*) gene.

https://gnomad.broadinstitute.org/region/19-48619291-48630717?dataset=gnomad r4

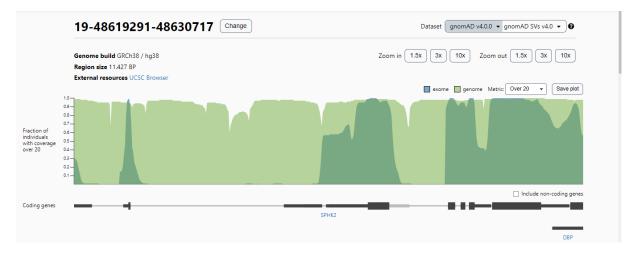


Figure 13. Gene Position for 19-48628944-T-C

b) REVEL and PolyPhen Scores

While REVEL score is 0.420, Polyphen (max) score is 0.998. While a REVEL score of 0.420 and a PolyPhen (Max) score of 0.998, this variant is associated with a lower likelihood of a disease according to REVEL. However, according to PolyPhen, it may signify a more impactful alteration in the protein, indicating a higher probability of pathogenicity.

In Silico Predictors

CADD: 25.9

REVEL: 0.420

SpliceAl: 0.00

Pangolin: -0.0100

phyloP: 6.25

PolyPhen (max): 0.998

Figure 14. In Silico Predictors.

c) ClinVar Informations

Not reported in Clinvar.

d) Variant Filter/Prioritize

The ClinVar data for the relevant variant is not available. However, by examining the REVEL and PolyPhen scores, we can suggest that there is a high likelihood of the variant being pathogenic.

Question 2. Protein Identification Through PMF (Peptide Mass Fingerprinting)

Firstly, we utilized the .txt file provided to us during the search stage on the Mascot page.

MASCOT Peptide Mass Fingerprint

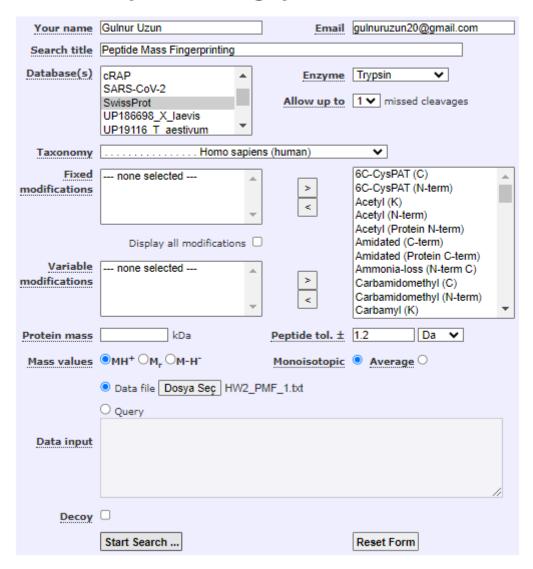


Figure 15. First Step on Mascot.

```
User : Gulnur Uzun
Email : gulnuruzun20@gmail.com
Search title : Peptide Mass Fingerprinting
MS data file : HW2_PMF_1.txt
Database : SwissProt 2023_05 (570420 sequences; 206321560 residues)
Taxonomy : Homo sapiens (human) (20429 sequences)
Timestamp : 17 Nov 2023 at 14:32:41 GMT
Top Score : 102 for HYEP_HUMAN, Epoxide hydrolase 1 OS=Homo sapiens OX=9606 GN=EPHX1 PE=1 SV=1
```

Figure 16. Displayed the name of protein.

a) The name of protein is Epoxide hydrolase 1 (Figure 16). e-value is **1.3e-06** for Epoxide hydrolase 1 protein (Figure 17). 1.3e-06 equal to 0.0000013, the e-value of the corresponding protein is a very small number, indicating that the likelihood of the match being random is quite low. The lower the e-value, the more significant the score.

Database: SwissProt
Score: 102
Expect: 1.3e-06
Monoisotopic mass (Mr): 52915
Calculated pI: 6.77
Taxonomy: Homo sapiens

Figure 17. Displayed the e-value.

b) In the relevant analysis, the number of peptides significantly matched to the protein is 35.

```
MS data file:
                    HW2 PMF 1.txt
Enzyme:
                    Trypsin: cuts C-term side of KR unless next residue is P.
Mass values searched: 135
Mass values matched: 35
 Start - End
               Observed Mr(expt) Mr(calc) Delta M Peptide
                                             0.0024 1 R.DKEETLPLEDGWWGPGTR.S
    22 - 39
               2085.9900 2084.9827 2084.9803
               1842.8500 1841.8427 1841.8584 -0.0157 0 K.EETLPLEDGWWGPGTR.S
    44 - 52
               1106.5700 1105.5627 1105.5404
                                              0.0223 0 R.EDDSIRPFK.V
    44 - 66
              2795.4000 2794.3927 2794.3158 0.0769 1 R.EDDSIRPFKVETSDEEIHDLHQR.I
    67 - 71
               678.3000 677.2927 677.3860 -0.0933 1 R.IDKFR.F
    67 - 71
               679.4100 678.4027 677.3860 1.0167 1 R.IDKFR.F
    92 - 98
               951.5300 950.5227 950.5338 -0.0110 1 K.KVISYWR.N
    93 - 98
                823.4300 822.4227 822.4388 -0.0161 0 K.VISYWR.N
                838.3700 837.3627 837.3657 -0.0030 0 R.NEFDWK.K
    99 - 104
    99 - 105
                966,4800
                          965.4727
                                    965.4607
                                              0.0121 1 R.NEFDWKK.O
                871.4900 870.4827 870.4923 -0.0096 0 K.QVEILNR.Y
   106 - 112
                691.2500 690.2427 690.3489 -0.1062 0 R.YPHFK.T
   113 - 117
   160 - 168 1009.6100 1008.6027 1008.6219 -0.0192 0 K.IIPLLTDPK.N
   197 - 206 1050.5900 1049.5827 1049.5618 0.0209 1 K.KGFNSVATAR.I
   198 - 206
               922.4900 921.4827 921.4668 0.0159 0 K.GFNSVATAR.I
   198 = 210 1474.7400 1473.7327 1472.7776 0.9551 1 K.GFMSVATARIFYK.L
   207 - 210
               569.2900 568.2827 569.3213 -1.0386 0 R.IFYK.L
   215 - 244
               3337.9700 3336.9627 3337.6264 -0.6637 0 R.LGFQEFYIQGGDWGSLICTNMAQLVPSHVK.G
               835.4600 834.4527 834.4599 -0.0072 0 R.FLOLTER.D
   271 - 277
   271 - 286 1892.0100 1891.0027 1891.0455 -0.0428 1 R.FLGLTERDVELLYPVK.E
   278 - 286 1075.6000 1074.5927 1074.5961 -0.0034 0 R.DVELLYPVK.E
   289 - 295
               915.4700 914.4627 914.4684 -0.0057 0 K.VFYSLMR.E
              1288.6100 1287.6027 1287.5884
                                              0.0143 0 K.FSTWTNTEFR.Y
   329 - 338
              1051.5300 1050.5227 1050.4982 0.0246 0 R.YLEDGGLER.K
   339 - 347
               2902.5500 2901.5427 2901.4946
                                              0.0481 1 R.KFSLDDLLTNVMLYWTTGTIISSQR.F
   349 - 372
               2773.6200 2772.6127 2773.3997 -0.7869 0 K.FSLDDLLTNVMLYWTTGTIISSQR.F
               1729.7900 1728.7827 1728.8294 -0.0466 1 R.FYKENLOQGWHTQK.H
   373 - 386
   376 - 386
              1292.6000 1291.5927 1290.6027 0.9901 0 K.ENLGQGWMTQK.H
   392 - 411
              2279.1900 2278.1827 2278.1674 0.0153 0 K.VYVPTGFSAFPFELLHTPEK.W
   415 - 419
               682.2900 681.2827 681.3850 -0.1023 1 R.FKYPK.L
   420 - 428
             1131.6000 1130.5927 1130.5794 0.0133 0 K.LISYSYMVR.G
                                             0.0028 0 R.GGHFAAFEEPELLAQDIR.K
   429 - 446
               1999.9900 1998.9827 1998.9799
               2128.0800 2127.0727 2127.0749
                                             -0.0022 1 R.OGHFAAFEEPELLAQDIRK.F
   448 - 454
               863.4900 862.4827 862.4912 -0.0085 0 K.FLSVLER.Q
               991.5700 990.5627 990.5498 0.0129 1 K.FLSVLERO.-
   448 - 455
```

Figure 18. Displayed the number of peptides significantly matched to the protein.

c) We displayed the amino acid sequence of the protein along with the matched peptides on the analysis page, highlighted in red. Protein sequence coverage is 58% for this sequence.

Protein sequence coverage: 58%

Matched peptides shown in bold red.

```
1 MWLEILITSV LGFAIYWFIS RDKEETLPLE DGWWGPGTRS AAREDDSIRP
51 FKVETSDEEI HDLHQRIDKF RFTPPLEDSC FHYGFNSNYL KKVISYWRNE
101 FDWKKQVEIL NRYPHFKTKI EGLDIHFIHV KPPQLPAGHT PKPLLMVHGW
151 PGSFYEFYKI IPLLTDPKNH GLSDEHVFEV ICPSIPGYGF SEASSKKGFN
201 SVATARIFYK LMLRLGFQEF YIQGGDWGSL ICTNMAQLVP SHVKGLHLNM
251 ALVLSNFSTL TLLLGQRFGR FLGLTERDVE LLYPVKEKVF YSLMRESGYM
301 HIQCTKPDTV GSALNDSPVG LAAYILEKFS TWTNTEFRYL EDGGLERKFS
351 LDDLLTNVML YWTTGTIISS QRFYKENLGQ GWMTQKHERM KVYVPTGFSA
401 FPFELLHTPE KWVRFKYPKL ISYSYMVRGG HFAAFEEPEL LAQDIRKFLS
```

Figure 19. Protein Sequence Coverage.

Question 3: Transcriptomic Data Analysis (Details in Excel file.)

Firstly, the t-test and fold change calculations have been performed in Excel using formulas.

For example IDP2 gene:

T.TEST(C6:J6; L6:S6; 2; 2)

#Explain the calculatio;

C6:J6 is aerobik range; L6:S6 is anaerobik range; 2: Specifies the use of an independent two-sample t-test; 2: Indicates that the variances of both samples are assumed to be equal.

For Fold Change (FC), averages for aerobic and anaerobic conditions were separately obtained, and then the FC was defined as the ratio of the aerobic mean to the anaerobic mean.

Typically, p-values less than 0.05 are considered statistically significant. The p-values and fold-change values have been obtained from the table provided to us. Below are the genes with the smallest p-values:

- For the IDP2 gene, the p-value is 1.01E-11, which is equal to 0.0000000000101246.
- For the PFK1 gene, the p-value is 2.03E-07, which is equal to 0.000000202763.
- For the CIT3 gene, the p-value is 2.02E-06, which is equal to 0.00000201516.
- The top 3 genes with the highest fold-change (FC) values are as follows:
- For the IDP2 gene, the FC value is 40.3443.
- For the CIT3 gene, the FC value is 22.36661.
- For the KGD2 gene, the FC value is 2.341349.

Based on these results, the data analysis and interpretation can be summarized as follows:

1. IDP2 Gene:

P-value: 1.01E-11 (very small)

FC: 40.3443 (high)

The p-value for this gene is very small, indicating statistical significance. Additionally, the high FC value suggests a substantial increase in gene expression. IDP2 has a higher FC compared to other genes, indicating a potentially significant role in biological processes.

2. PFK1 Gene:

P-value: 2.03E-07 (very small)

o FC: 2.03E-07 (low)

The p-value for PFK1 is statistically significant. However, the low FC value suggests a more limited change in gene expression, potentially resulting in a lower biological impact compared to other genes.

3. CIT3 Gene:

P-value: 2.02E-06 (very small)

FC: 22.36661 (high)

The small p-value and high FC value indicate a significant increase in gene expression for CIT3. This gene may play a crucial role in biological processes due to the noteworthy increase in expression.

4. KGD2 Gene:

P-value: Not providedFC: 2.341349 (moderate)

Although the p-value is not specified, the moderate FC value suggests a noticeable change in gene expression, albeit to a lesser extent compared to other genes.

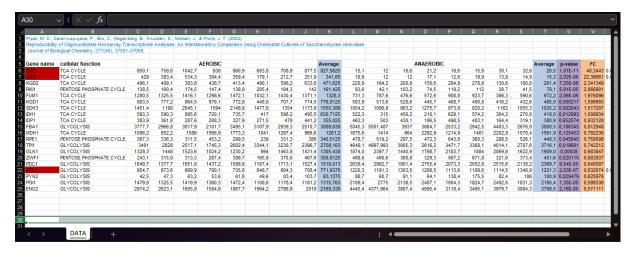


Figure 20. Displayed Excel File.

Question 4: The answer to this question is found in the .R file.

Figure 21. R Script for Question 4.

Figure 22. Outputs for R Script (Question 4).