Lab Report: PTC Lab

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Abstract:
The TAS2R38 gene allows certain individuals to taste the molecule Phenylthiocarbamide (PTC). The T allele of this gene allows individuals to taste PTC, while the t allele causes the individual to not be able to taste PTC at all. Three single nucleotide polymorphisms can help predict overall which of the two alleles a given individual has. A heterozygous individual is able to taste PTC, but only slightly. The research in this paper determines if the allele frequency of the TAS2R38 gene in immigrants matches up with the allele frequency of the country that they immigrate to (in our case, the US). We ran both a general phenotype test and a genotype test utilizing PCR, a digestion enzyme, and gel electrophoresis on a group of 18 subjects from our class, who were mostly immigrants from other countries. We observed that there were 5 strong-tasters (phenotype TT), 10 weak-tasters (phenotype Tt), and 3 non-tasters (tt). Using the Hardy-Weinberg principle, we determined that the subjects had a 60% frequency of the T allele, and 40% frequency of the t allele. Previous research shows that the US had a 90% frequency of the T allele and a 10% frequency of the t allele.[3] This meant that the frequencies did not match up, though we also note that the sample size is so small that we were unable to come to a complete conclusion. However, we hypothesized that it shows how gene flow promotes the genetic diversity of the place that individuals migrate to.

Introduction:
Phenylthiocarbamide (PTC) is a bitter-tasting compound that certain portions of the world population are able to taste. The PTC taste receptor gene (TAS2R38) is the gene that codes for the receptors for PTC. It can have single-nucleotide polymorphisms at the 145, 785, and 886 base pair positions, and code for amino acids 49, 262 and 296.[1] The polymorphisms cause the non-tasting allele to form, which is recessive and heritable through Mendelian Genetics.[2] The three genotypes for the TAS2R38 gene is TT, which codes for a strong reaction to tasting PTC; Tt, which codes for a weak reaction to tasting PTC; tt genotype, which codes for no reaction to PTC.

Even though PTC is a synthetic substance, there are certain natural toxic compounds produced by plants as a defense mechanism (such as strychnine, quinine, and ricin) that only people with dominant alleles of TAS2R38 can taste.[2] This means that people who have dominant alleles of the gene have an advantage in areas where the toxic compound

is common, and that is indicative of natural selection. There is variation within the population, as there are two alleles, the trait is heritable, and there is a clear fitness advantage for people have the dominant allele of PTC (as people with the recessive genotype would be unable to taste the compounds and thus be harmed by the plant's toxins) This advantage indicates that studying the frequencies of the TAS2R38 alleles can provide an understanding of how the people in different regions evolved with this gene.

Previous research on TAS2R38 shows that certain areas of the world have higher expression of the dominant allele, and are able to taste PTC. Most notable is that the frequency of the dominant allele is higher in Europe, while it is lower in Asia and Africa.[2] However, in recent years, immigration has become more accessible and common, so people of various descents can easily move from country to country. Particularly, the subjects of this experiment – the students in our class – are immigrants; the goal of this lab report is to determine if the subjects' TAS2R38 allele frequencies match the allele frequencies of where they were born, or where they live now (the US). Hence, the research question of this lab report is, "Do the subjects' PTC receptor allele frequencies reflect the previously researched allele frequency distributions for TAS2R38 in the United States?" The hypothesis of this paper is that the allele frequency distribution will not reflect that of the US because the subjects' varied backgrounds mean that the allele frequencies will be representative of those found in the subjects' birthplaces.

Materials & Methods:

Test 1 (PTC phenotype paper test):

Materials

- Control test paper (no PTC)
- PTC-containing paper

Procedure

1. Have every test subject take a control paper
2. Have each test subject touch the control paper to the tip of their tongue
3. Give every test subject a PTC-containing test paper
4. Have each test subject touch the PTC-containing test paper onto the tip of their tongue, and ask them to note if there is a strong, weak, or no bitter taste
5. Record results

Test 2 (TAS2R38 genotype test):

Materials

I. Isolate DNA by Saline Mouthwash

A. Reagents

- Saline solution ($NaCl+H_20$)

- Chelex®

B. Supplies and Equipment
- 1.5 ml microcentrifuge tube
- Permanent marker
- Micropipet (10-1000 µL)
- Micropipet tips (10-1000 µL)
- Microcentrifuge, tube racks, and adapters
- Hot plate
- Ziploc bag with ice

II. Amplify DNA by PCR

A. Reagents
- Cheek cell DNA, from part I
- PCR primer/loading dye mix
- Ready-to-go™ PCR bead

B. Supplies and Equipment
- Permanent markers
- Micropipet (1-100 µL)
- Micropipet tips(1-100 µL)
- Microcentrifuge tube rack
- PCR Machine
- Ziploc bag with ice

III. Digest PCR Products With *Hae*III

A. Reagents
- *PCR product (from Part II), 25 µL

B. Shared Reagent
- *Restriction enzyme *Hae*III, 10 µL

C. Supplies and Equipment
- Permanent Marker
- 1.5-mL microcentrifuge tubes
- Microcentrifuge tube rack
- Micropipet and tips (1-20 µL)
- Thermal cycler (or water bath or heat block)
- Container with cracked or crushed ice

IV. Analyze PCR Products by Gel Electrophoresis

A. Reagents
- Undigested PCR product (from Part III), 10 µL
- *Hae*III-digested PCR product (from Part III), 16 µL

B. Shared Reagents
- pBR322/*Bst*NI marker
- Pure Agarose

- 1xTBE, 300 mL
- Sybr Safe gel dye

C. Supplies and Equipment
- Micropipet and tips (1-20 µL)
- Microcentrifuge tube rack
- Gel electrophoresis chamber
- Power supply
- Staining trays
- Latex gloves
- UV transilluminator (for use with *Carolina*BLU)
- Digital or instant camera (optional)
- Water bath (60 ℃)
- Container with cracked or crushed ice

Procedure

Part I: Isolate DNA By Saline Mouthwash

1. Label a 1.5 mL tube with your assigned identification number.
2. Rinse your mouth with the saline solution and spit it into the assigned tube.
3. Gently swirl the tube to mix the settled cells.
4. Place the sample tube into a microcentrifuge for 90 seconds at full speed.
5. Extract 960 µL of the supernatant using a micropipette.
6. Resuspend the cells by setting a micropipette to 30 µL and pipetting the solution in and out.
7. Use a micropipette to add 100 µL of Chelex® to the tube.
8. Place the 1.5 mL tubes into a boiling water bath for 10 minutes at approximately 100ºC.
9. Shake the tube for 5 seconds.
10. Place the tube into a microcentrifuge and spin for 90 seconds at full speed.
11. Store the tube in ice until you are ready for Part II.

Part II: Amplify DNA by PCR

1. Place a Ready-To-Go™ PCR bead into a PCR tube and label the tube with your assigned number.
2. Add 22.5 µL of PTC primer/dye mix to the PCR tube and allow the bead to dissolve into the mix.
3. Add 2.5 µL of cheek cell DNA from Part I into the loading dye mix.
4. Store the sample in ice until you are ready for the PCR process.
5. Place the PCR tube in a thermal cycler for 30 of the following cycles.
   - Denaturing Step: 94ºC for 30 seconds
   - Annealing Step: 64ºC for 45 seconds
   - Extending Step: 72º for 45 seconds

6. Store the amplified DNA in ice until you are ready to continue to Part III.

Part III: Digest PCR Products with *Hae*III

1. Label 1.5 mL tube with your assigned number and a "U" for undigested mixture.
2. Transfer 10 µL of PCR product into the "U" tube.
3. Use a micropipette to add 1 µL of Restriction enzyme *Hae*III directly into the PCR tube, and label the tube "D" for digested mixture.
4. Mix these two reagents with a microcentrifuge.
5. Place the PCR tube into a thermal cycler programmed to hold 37ºC for 30 minutes.
6. Store the sample in ice until ready for Part IV

Part IV: Analyze PCR Products by Gel Electrophoresis

1. Seal and set up the gel-casting tray, and insert a comb to form the wells.
2. Mix 2 grams of agarose and 100 mL of 1xTBE, heating it up until it forms a clear solution.
3. Add 10 µL of Sybr Safe into the solution.
4. Pour the solution into the tray.
5. Remove the comb once the solution has solidified.
6. Pipette the PCR solutions, both undigested and digested into the wells.
7. Run the gel for 30 minutes at 130 Volts.
8. Observe the gel and collect results.

Results

Test 1 (the phenotype paper test) showed that 8 subjects were strong-tasters, 5 were weak-tasters, and 3 were non-tasters. These results are shown in Figure 1 below.

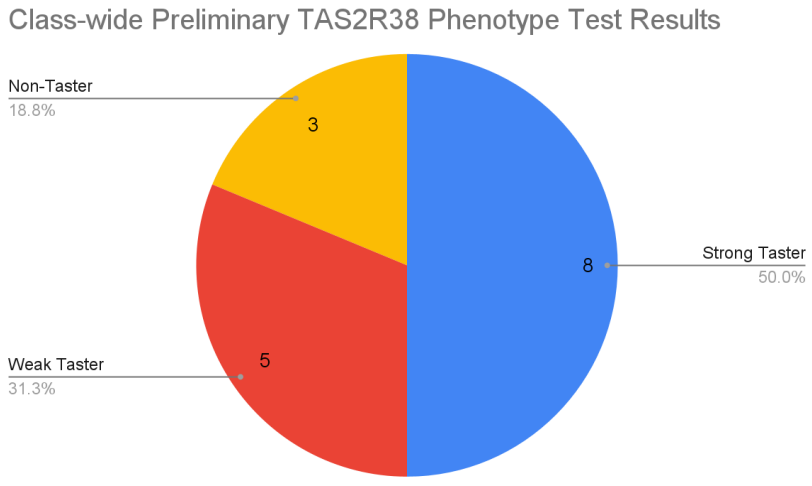

Figure 1: The Qualitative Results for Test 1 Phenotype Predictions. Figure 1 represents the ratios of the subjects' non-taster, strong-taster, and weak-taster phenotypes. 47.1% were predicted to be strong-tasters, 17.6% were predicted

to be non-tasters, and 35.3% were predicted to be weak-tasters. It should be noted that not every subject took the phenotypic test; the population size here is 16, while the population size for the genotype measurements is 18.

Test 2 (testing genotypes for the TAS2R38 gene) produced two trays of gels as shown in Figures 2 and 3**.**

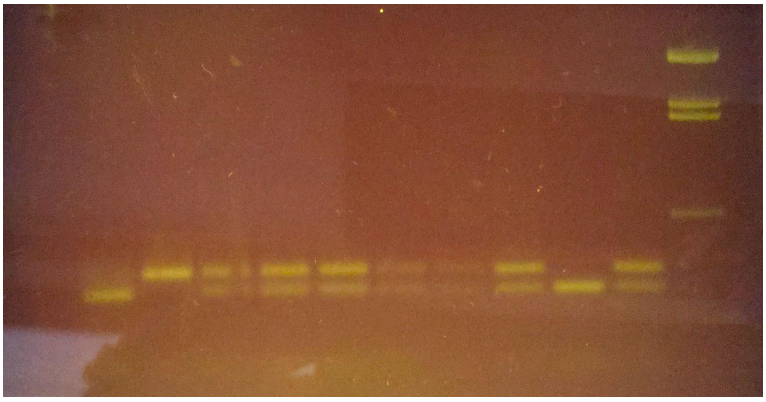

Figure 2: Results of Digested Samples Gel Electrophoresis, Tray 1. From left to right: DNA Sample 1 (2 bands), DNA Sample 2 (1 band), DNA Sample 3 (3 bands), DNA Sample 4 (3 bands), DNA Sample 5 (3 bands), DNA Sample 6 (3 bands), DNA Sample 13 (3 bands), DNA Sample 14 (3 bands), DNA Sample 15 (12 bands), LW DNA Sample (3 bands), Marker.

In Figure 2, each band represents the DNA strands that were of a given base pair length, which was determined either by estimation or using a ladder (shown far left of Figure 3 below). The bands further down the gel traveled further because the DNA strands were of shorter length. The brightness of the band indicates a higher concentration of the DNA of that length present. Three primary rows/sections are shown in the gel. The first row, on the top, represents DNA of length 221 bp, the second row represents 177 bp DNA, and the final row represents 44 bp DNA, which is very faint.

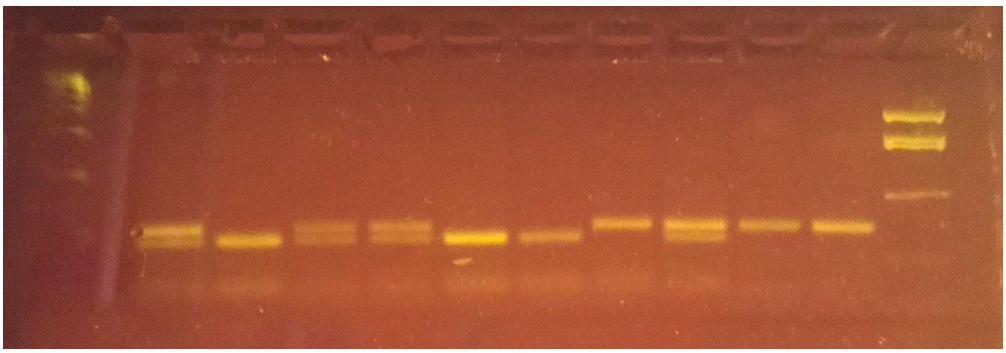

Figure 3: Results of Digested Samples Gel Electrophoresis, Tray 2. From left to right: Ladder, Sample 7 (3 bands), Sample 8 (2 bands), Sample 10 (3 bands), Sample 11 (3 bands), 12 (2 bands), Sample 17 (2 bands), Digested DNA 19a (1 bands), Digested DNA 20 (3 bands), CV DNA Sample (1 band), CV DNA Sample (1 band)

In Figure 3, just like in Figure 2, the first row from the top represents 221 bp length DNA, the second represents 177 bp DNA, and the third represents 41 bp DNA.

In total (between trays 1 and 2), there were 5 samples with 2 bands, 10 samples with 3 bands, and 3 samples with 1 band.

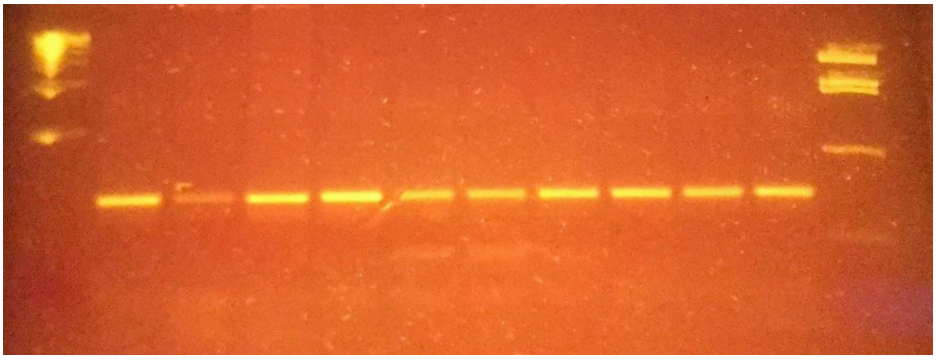

Figure 4: Results of Undigested DNA Gel Electrophoresis, Tray 3. Figure 4 depicts the gel electrophoresis for the undigested samples of DNA

There were two trays (trays #3 and #4) of undigested DNA. However, both are identical and all have bands at roughly the same place. Hence, only tray #3 is shown.

Discussion

In this paper, it was tested whether the subjects' TAS2R38 allele frequencies reflected the previously researched allele frequencies for the US. This was done in order to observe the effect of immigration and travel on the allele frequencies in the new countries.

The first test was the paper phenotype test. However, a limitation of this is that taste is very subjective, which means it can be difficult to determine whether someone is a strong or weak-taster. Doing Test 2 on the subjects' DNA to get quantitative results about the subjects' genotypes and phenotypes was a follow-up to this. Test 1 can also be seen as a control, because if the determined genotype and phenotype for a subject in Test 2 is drastically different from what they determined through Test 1 (for example, if the subject could strongly taste PTC but their genotype was tt), the Test 2 results would be inaccurate.

The *HaeIII* primer was set to specifically cut the T allele of the TAS2R38 gene (which is 221 bp long) into two strands, 44 and 177 bp long, as it would only recognize and splice the DNA fragment if the SNPs for the T allele were present (It recognized the sequence 5' GGCC 3', which is only present in T allele). This means that if there were two bands, the genotype would be *TT*, since both alleles would be cut and thus would produce only the two bands at 177 and 44 bp. If the genotype were tt, there would only be one band (since the *HaeIII* enzyme would not cut the t allele, preserving the 221 bp length). If the genotype was Tt, there would be three bands, because the T allele in the genotype would be cut and produce two bands, while the t allele (which is not cut and will have full length) would produce another, for a total of three bands. While the 44 bp band is

difficult to observe on the images shown, it is always present when the 177 bp band (the middle one since smaller bands travel further in a gel) is present. None of the results were false positives, as the undigested DNA indicated that all the undigested DNA samples had the same length on the graph. The undigested DNA was a control group because it showed that none of the DNA had been unintentionally cut during the PCR step. Thus, the differences in length of DNA can only be explained by the *HaeIII* enzyme. Adding up all of the different genotypes observed in the gel, there were 5 strong-taster phenotypes (TT), 10 weak-taster phenotypes (Tt), and 3 non-tasters (tt). Expressing this data as a pie chart, the ratio between the different genotypes can be seen:

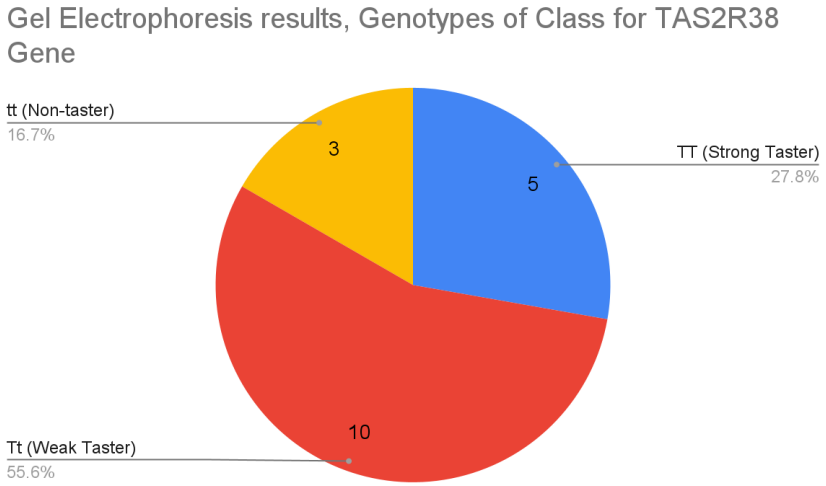

Figure 5: The Quantitative Results for Gel Electrophoresis. Figure 5 represents the ratios of the non-taster, strong-taster, and weak-taster genotypes. 27.8% of the subjects were strong-tasters and had genotype TT, 55.6% were weak-tasters and had genotype Tt, and 16.7% were non-tasters and had genotype tt.

Figure 5 shows that 5 subjects had the strong-taster phenotype (TT), 10 had weak-taster phenotype (Tt), and 3 had non-taster phenotype (tt) for PTC. This information, along with the Hardy-Weinberg equilibrium, can be used to find the frequencies of the alleles for the TAS2R38 gene. The Hardy-Weinberg equilibrium states that the frequencies of the alleles can be found using the frequencies of the genotypes with the equation $p^2 + 2pq + q^2 = 1$, where p^2 is the frequency of the homozygous dominant genotype, $2pq$ the frequency of the heterozygous genotype, and q^2 is the frequency of the homozygous recessive genotype. The variables p and q represent the frequencies of the dominant and recessive alleles, respectively, and these also have a relationship, in that $p + q = 1$. The homozygous recessive genotype (q^2) has a frequency of 3/18, or $0.1\overline{6}$. The square root of that value - approximately 0.408 - is the frequency of the recessive allele (q). The frequency of the dominant allele (p) will be the frequency of the recessive allele subtracted from 1 ($1 - q = p$), which is 1-0.408=0.592. This can then be compared to Wooding et al.'s observed frequencies of the alleles for TAS2R38 (shown

below). It is important to note that one of the assumptions the Hardy-Weinberg principle makes is that the population size used is large. Since the subject population size is extremely small, using this principle could provide inaccurate results. However, it still serves as a good approximation for the allele frequencies examined here.

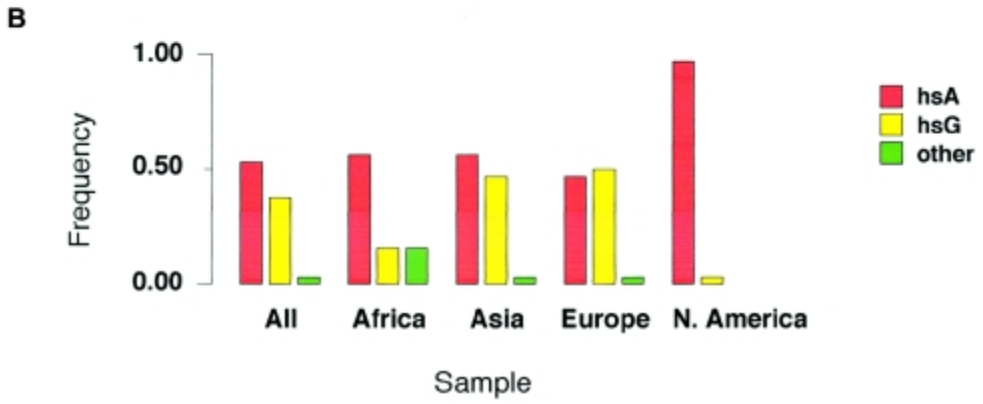

Figure 6: Allele frequencies for different world regions.[3] Figure 6, created in a research study by Wooding et al., shows the allele frequencies of the T (hsA) and t alleles (hsG) for the TAS2R38 genes in five regions, the world (All), Africa, Asia, Europe, and North America.

The data in Figure 6 shows that the taster allele (T) has approximately 90% frequency in the North American region, while the non-taster allele (t) has less than 10% frequency in North America. These frequencies are not reflected in this experiment's results. Instead, the results more closely resemble the "All" data, which has a roughly 60% taster allele frequency, and a 40% non-taster allele frequency. This means that the experiment's data does not reflect the allele frequencies within the North American regions; instead, it conforms to the general world frequency. So, the original hypothesis was correct, and the subjects' allele frequencies do not line up with the US. However, there are very few subjects in the testing group, making it difficult to make generalizations about all immigrants. For just the subjects used for this experiment, the frequencies of these immigrants from Figure 6 do not line up with the US allele frequency data.

There is evidence here of how gene flow can promote the genetic diversity of a population, that being the United States. The subjects, mostly made up of immigrants or direct descendents of immigrants, did not match up with the previously researched US allele frequency, but since it is now inside the US, the people in it are part of the gene pool. Given the high allele frequency of the taster allele in the North American region, immigrants from countries where the frequency of the non-taster allele has increased will then create more genetic diversity in the US (more varied allele distributions) by adding their non-tasing alleles to the US gene pool. Since immigration has been made much easier in recent decades, this then means that the allele frequency of the non-taster allele

and genetic diversity in the US can continue to rise, as citizens of other countries bring the non-taster allele to the US through immigration.

A limitation of this experiment, as previously mentioned, is the small sample size, because the 18 samples taken are not enough to make a conclusive statement about all immigrants and their allele frequencies. A follow-up experiment would be to run the paper test experiment for a few hundred people in Seattle, and ask them whether they and their parents were immigrants, or were born in the United States. Using this data it would be possible to calculate their genotypes and assess whether the subjects whose parents were born in North America had similar allele frequencies to the given data in Figure 6. This larger sample size will provide a more conclusive statement about allele frequencies of the taster gene. Additionally, this experiment can be extended by analyzing more specific categories of immigrants, like people from Europe, South America, Asia, and Africa. This will allow more specific analysis on whether immigrants' genotypes reflect their specific home country's allele frequencies, or the US.

Conclusion:

The research in this paper determined that the class had an 60% allele frequency for the taster allele (T) and a 40% allele frequency for the non-taster (t) allele. This did not match up with the previously researched allele frequency for the US (90% *T* allele), and that led to the conclusion that the subjects who are immigrants were not well represented by the allele frequencies in the US. The original question, however, had to stay unanswered, since nothing could be determined about all immigrants given the limited sample size. If the subjects were somewhat representative of the immigrant population in the US, that shows that immigrants (with their different allele distributions) would promote diversity of alleles in the new regions, proving how gene flow benefits genetic diversity.

References

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