Singular Nucleotide Polymorphisms in Genes that Represent Photic Sneezing and PTC Tasting Traits

Kaleigh Beale

Biology 250

Spring 2020

**Abstract**

In this experiment, single nucleotide polymorphisms are examined for two different traits for one individual. The traits tested were photic sneeze reflex which is found at locus rs10427255 and Phenylthiocarbamide (PTC) or bitter tasting trait found at locus rs713598. Photic sneeze reflex is seen in people who sneeze after being exposed to high intensity light and PTC is a compound that tastes extremely bitter to people who have the trait and tasteless to those without it. The purpose of this experiment was to determine if the DNA samples were homozygous or heterozygous for a particular trait. The samples were collected by swabbing the inside of the individual’s cheek and putting the cheek cells in a container of sterile water. The samples underwent Polymerase Chain Reaction (PCR), gel electrophoresis, purification, and DNA sequencing. The results showed that the sample for photic sneeze reflex, sample 9C1, had one peak representing cytosine at nucleotide 226 and the sample for PTC (bitter) tasting, sample 9C2, had two peaks representing cytosine and guanine at nucleotide number 449. While it was initially hypothesized that both samples would be heterozygous, the results rejected the hypothesis in the sense that sample 9C1 was found to be homozygous for the photic sneezing reflex trait and sample 9C2 was heterozygous for the PTC (bitter) tasting trait.

**Introduction**

Single Nucleotide Polymorphisms (SNPs) are a common type of genetic variation among humans. Each SNP represents a difference in a single nucleotide. For example, cytosine may be replaced by thymine or guanine which would cause a change in the expression of the gene that is being coded for. These genetic variations have been linked to certain traits or diseases like Crohn’s Disease (D’Addabbo, 2019).

In this experiment, two singular nucleotide polymorphisms were investigated, SNP1: Photic sneezing reflex and SNPB: PTC (bitter) tasting. The photic sneezing reflex has been found at locus rs10427255 and rs1032507 (Wang, 2019). However, we only examined the locus rs10427255. The PTC tasting gene was located on locus rs713598 (Perna, 2018).

Generally, sneezing a protective measure that expels particles from the nasal cavity. However, photic sneeze reflex (PSR) is an uncontrollable reflexive sneeze in response to sudden exposure to bright light. Depending on the ethnicity of a subject, the SNP is likely to vary. In U.S. populations cytosine contributed to a significantly higher risk for PSR but in China the nucleotide was Thymine (Wang, 2019).

There are approximately 30 taste receptors for bitterness in the human mouth and the ability to taste bitterness varies based on the activity of these receptors. Phenylthiocarbamide (PTC) is an organosulfur thiourea containing a phenyl ring which binds to one of the bitterness receptors. The ability to taste it is controlled by a gene at the locus rs713598. It seems to follow a Mendelian recessive manner where the inability to taste PTC is recessive. The ability to perceive bitter taste has a strong influence on food consumption. People who are able to taste bitterness tend to eat bitter foods more infrequently and cause them to miss out of the antioxidants those foods contain (Perna, 2018).

The purpose of this experiment was to determine if the DNA samples were homozygous or heterozygous for a particular trait. Chromatograms for each DNA sequence are to be analyzed to determine the results. One peak at the SNP location signifies the gene is homozygous, while two peaks signify that the gene is heterozygous. It was hypothesized that both traits will be heterozygous. The importance of the study mainly relates to the individual tested. It will provide the individual an understanding of their food choices and determine whether light is a factor that induces sneezing for them.

**Methods and Materials**

Students swabbed their checks and put their cheek cells into a container with sterile

water. Five microliters of the cheek cell solution and the corresponding primer mix were added to a PCR tube, along with 25 microliters of Phire Tissue Direct PCR Master Mix (Invitrogen) and 15 microliters of sterile water. The primers for photic sneeze reflex were Forward: CATTATTGTGCAGGTCTGCGATT and Reverse: TTTGCAATATGCCTGGCAATGA. The primers for PTC (bitter) tasting were Forward: CTGTGAAGTGAGGTCTGCTAAA Reverse: ATCCGTGATGCTGTGCTATG. The master mix included Taq DNA polymerase, a buffer for salt and pH, and dNTPs. Once the everything was placed in the tube, a polymerase chain reaction was run according to Table 1.

|  |  |  |
| --- | --- | --- |
| **Step** | **Temperature** | **Time** |
| Initial Denaturation | 98°C | 5 Minutes |
| 30 Cycles | 98°C  55°C  72°C | 5 Seconds  5 Seconds  20 Seconds |
| Final Extension\* | 72°C | 1 Minute |
| Hold | 4°C |  |

**Table 1.** PCR thermocycling profile.

\*The final extension for SNPB (PTC Tasting) was held at 62°C for 30 seconds

A gel electrophoresis chamber was set up to include a 2% agarose gel mold and 0.25X TAE buffer. The PCR products and a 100bp DNA ladder were placed into wells of the gel mold. Gel electrophoresis was performed at 300V for 15 minutes.

The PCR reaction samples were added to a tube containing 250 microliters of the corresponding Binding buffer and was mixed well. This solution was then transferred to a spin filter column and centrifuged for 1 minute at 13,000 rpm. The Binding buffer and PCR reaction components that passed through the filter and were discarded. Two hundred microliters of DNA Wash Buffer were added to the spin filter column and it was centrifuged for 1 minute at 13,000 rpm. The wash buffer pushes contaminants through the filter like proteins and salts which were also discarded. The DNA was washed once more with 200 microliters of DNA Wash Buffer to remove all remaining contaminants. The spin filter column was then inserted into a sterile microcentrifuge tube. Thirty microliters of the elution buffer were added to the center of the white filter and was allowed to sit for 1 minute so the elution buffer would rehydrate and suspend the DNA. The sample was centrifuged, once more, at 13,000 rpm for 1 minute and the DNA was collected in the microcentrifuge tube. The nanodrop was used to measure the DNA concentration (ng/microliter) and A260/A280 values of 2 microliters of the purified product.

Ten microliters of the purified amplicon samples were placed into a sequencing reaction tube containing the primer used in the amplification step. The tube was sent to Eurofins Genomics for sequencing. The sequences were analyzed by comparing the experimental data to the known sequences and the SNPs and primers were identified.

**Results**

The experimental results showed that sample 9c1 had 326 base pairs and sample 9c2 had 1030 base pairs. This was calculated by measuring the migration of the samples, as shown in figure 1.



**Figure 1.** Gel electrophoresis

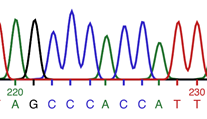
Sample 9c1 was found to have an absorbance of 0.104 at 260 nm. The A260/280 ratio was 1.84 and there was a DNA concentration of 5.2 ng/microliter. Sample 9c2 had an absorbance of 0.109 at 260 nm. The A260/280 ratio was 1.51 and there was a DNA concentration of 5.4 ng/microliter.

The DNA sequences for sample 9C1 and 9C2 are shown in figure 2. The SNP for sample 9C1 was found to be cytosine, while the SNP for sample 9C2 was guanine.

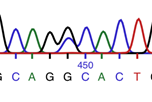
|  |  |  |
| --- | --- | --- |
|  | Experimental DNA Sequence | Known DNA Sequence |
| Photic Sneeze Reflex gene for Sample 9C1 | NNNNNGCTTCTGTGANGGTGANGCTGCCTGTCAGCAGACCATTCTTTGTGTAGAAAGATTGTACACNGGTTAACAAAAAAAAACAGTAATTGAAAGAATAATTCCATGTGTCAATAGATCAATAGAACAGAATGGAGTGATGAAAAAGGGGTCCTAATATAATGAGAACTTAGTAAATAATAAAGGTAGATATTTTCATCAATGAGGAAAGAAGGATTTAGCCCA**C**CATTAGTGTTGAGGAAATGGATCAGATCTTTGATGCAAAGAATATCATCTTCCTCTTTCACATCAATACTAAAGTCATTGCCAGGATAATTGCAAAAANN | TCATGGCTGCATAGTATTCCATGGTGTATACGTACCACATTTTCTTTACCCAGCCTATTATTGATGGGTATTTGGGTTGTTTCCATGTCTTTGCTACTGTGAATAGTGTTGCAATGAACATATGCATGCATGTATTTAACTTTGATATGCATAGGAATAATGTAGAGACCATTATTGTGCAGGTCTGCGATTGATTCTTCATTTCTAACATGCTTCTGTGAGGGTGATGCTGCCTGTCAGCAGACCATTCTTTGTGTAGAAAGATTGTACACAGGTTAACAAAAAAAAACAGTAATTGAAAGAATAATTCCATGTGTCAATAGATCAATAGAACAGAATGGAGTGATGAAAAAGGGGTCCTAATATAATGAGAACTTAGTAAATAATAAAGGTAGATATTTTCATCAATGAGGAAAGAAGGATTTAGCCCA**C**CATTAGTGTTGAGGAAATGGATCAGATCTTTGATGCAAAGAATATCATCTTCCTCTTTCACATCAATACTAAAGTCATTGCCAGGCATATTGCAAAATCTTGTGTAAAGTTCAATACTATAAAAAAGTCAAAGCATAAAACTATAAAATAAGCATAAACTCAGAATGGGGCGTAATTTAAAGTATAAAACAAAGGCAAAACTACAAAGGATAACAATTGATATATCTGACAAAATACACATAAACTTTTTTATGTTAAAATAATTGTGAGGTAAATAAGTTATGAACATATTTACAGAGTGTAACATTTCTAGTAAAAAATTGATTATTGAAAGTTTCTATCATATGACTCAGTAATTCTACTTATTTTAGCAAAATATTCCAAGATGTGTATAAAGGTATTTTCATATAGATATGAACCATAGCATAAATGAGTATATTAA |
| PTC (Bitter) Tasting gene for Sample 9C2 | GTNNNACACCTGGANTTATTAATCTTGCATCACCCAAGAGGTAGAACCATCAGTCTTCCACCCTATGATAAGCTCTTACGTGTATCCAAGAGATGTTCTAGAGAAACAACATCCCTCTAAGTTTCCTGCCAGAACTTTTTATGCGCTCGCTTTGGGATAGATCTAGGCAAAGAGCTGGATGCTTTGTGAAGGAAAGGTCCTGGCTTGGAACGTACATTTACCTTTCTGCACTGGGTGGCAACCAGGTCTTTAGATTAGCCAACTAGAGAAGAGAAGTAGAATAGCCAATTAGAGAAGTGACATCATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCAG**G**CACTGAGCAACAGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTCCTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCACTGAACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTTGCTGCCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTGGCAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGCACTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGNNNNNNNNTTNNNAGAANN | GTTGTGACTGTGAAGTGAGGTCTGCTAAAAAAGCACCAAACACAGAGGACAGTGCAGATGCAGGAGCAAAGAATAATACCCAGGAGCATCTGGGAGATCTTCCTGGAGACCCAGCTTGCCAAGCAGATCAGGAAGGTGTGAGAGAAACGGATGAGCTTGGAGCAGTAAAGCAGGCTGAGGCAGGCAGCAAGCCAGAGGTTGGCTTGGTTTGCAATCATCCATAGCATGATGATGGCTTGGTAGCTGTGGTTCAGTGGTTCACTCAACTTCTGGAAGTGGGTAAGCTGGATAGCACTCAGGAACAGCAGTCCATGCAGGAAAAGCCGGCTGATGCTGAGACACAGCAGCACACAATCACTGTTGCTCAGTG**C**CTGCCTCTTCACTACATCCCAAAAATTCACCAAGAAAACGAAGGCATTGGTCAGAAACCCCACTGCAAACTCCAGGACTGAAATGAACAGAAATGTACTCCTGACTTCATAGGACACAGTGCGGATGCGAGTTAGAGTCAACATGATGTCACTTCTCTAATTGGCTATTCTACTTCTCTTCTCTAGTTGGCTAATCTAAAGACCTGGTTGCCACCCAGTGCAGAAAGGTAAATGTACGTTCCAAGCCAGGACCTTTCCTTCACAAAGCATCCAGCTCTTTGCCTAGATCTATCCCAAAGCGAGCGCATAAAAAGTTCTGGCAGGAAACTTAGAGGGATGTTGTTTCTCTAGAACATCTCTTGGATACACGTAAGAGCTTATCATAGGGTGGAAGACTGATGGTTCTACCTCTTGGGTGATGCAAGATTAATAAATCCAGGTGTTCCTGTACAACCGCCTTCCCAGAAGGCCATAGCACAGCATCACGGATGCAAACA |

**Figure 2.** The experimental and known DNA sequences for Sample 9C1 and 9C2 are shown above. The SNP is shown in red text and the primers are highlighted in green.

The SNP for photic sneeze reflex, shown in figure 3, was found at nucleotide number 226. There was one peak and it was for the cytosine nucleotide. The SNP for PTC (bitter) tasting, shown in figure 4, was at nucleotide number 449. There were two peaks. The higher peak was guanine and the lower was cytosine.



**Figure 3.** DNA sequence and SNP1 for sample 9C1 was found at nucleotide 226.



**Figure 4.** DNA sequence and SNPB for sample 9C2 was found at nucleotide 449.

**Discussion**

The aim of this study was to determine whether the genes were homozygous or heterozygous. The results showed that there was one peak present for SNP1 (figure 3) which indicates that the photic sneeze reflex gene is homozygous for cytosine. While figure 4 showed there were two peaks present for SNPB: PTC (bitter) taste indicating that it is heterozygous. Therefore, the initial hypothesis that both SNPs would be heterozygous was rejected.

One limitation of this study is that only one sample for each SNP was run. Without testing multiple samples, it is unclear whether the study is accurate or not. An extension of this experiment would to test the SNPs again but with more samples from individuals with different phenotypes and backgrounds. This will ensure that the testing is accurate and that it is not specific to one ethnicity (Wang, 2019).

Additionally, this experiment should be applied to different loci in order to get a broader understanding of how the results translate to phenotypes. In a study by *Kim et al* (2003), it was shown that there are many different loci that code for the PTC trait. While they studied three different loci on the same chromosome, there were different results among individuals in different states and with different backgrounds (Kim, 2003).

**References**

D’Addabbo, A.; Latiano, A.; Palmieri, O.; Maglietta, R.; Annese, V.; Ancona, N. 2007. Regularized least squares classifiers may predict Crohn’s disease from profiles of single nucleotide polymorphisms. *Annals of Human Genetics* *71*, no. 4 (2007): 537-549

Kim, U.; Jorgenson, E.; Coon, H. et al. 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science,* 299(5610), 1221-1225

Perna, S.; Riva, Nicosanti, Carrai, Barale, Vigo, Allegrini, Rondanelli, 2017. Association of the bitter taste receptor gene TAS2R38 (polymorphism RS713598) with sensory responsiveness, food preferences, biochemical parameters and body-composition markers. A cross-sectional study in Italy. *Int J Food Sci Nutr.* 69(2):245-252

Wang, M.; Sun, X.; Shi, Y.; Song, X.; Mi, H. 2019. A genome-wide association study on photic sneeze reflex in the Chinese population. *Sci Rep*. 9(1):4993.