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Identification of Human Polymorphisms in the Phenylthiocarbamide (PTC) Bitter Taste Receptor Gene and Protein

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This paper introduces a lab project developed for the Summer Teachers' Workshop in Genomics at Amherst College, and is easily tailored to the weekly format of undergraduate laboratory courses in genetics, genomics, molecular biology, or evolution. The project examines single nucleotide polymorphisms associated with human taste sensitivity to the bitter compound phenylthiocarbamide (PTC). Human cheek cell DNA is amplified and sequenced using PTC-specific primers, and sequence variations in the PTC gene are correlated with taste sensitivity to PTC strips. A 'dry lab' version of the activity can also be done using pre-obtained DNA sequences from individuals with known PTC genotypes.

Keywords: bitter taste receptor, SNPs, phenylthiocarbamide (PTC)

Introduction

In this project, group members investigate the association in different people between taste sensitivity to the bitter compound phenylthiocarbamide (PTC) and single nucleotide polymorphisms (SNPs) in the PTC bitter taste receptor gene (*PTC*; also known as *TAS2R38*, for taste receptor, type 2, member 38). The inability to taste certain compounds has long been believed to be due to simple recessive Mendelian inheritance, and a large number of taste and odorant receptors have been cloned and sequenced in the last twenty years to establish the molecular basis of these traits (see references 23-24 in Kim *et al*., 2003). Kim and co-workers (2003) identified a region on chromosome 7 with strong linkage to PTC taste sensitivity. Interestingly, this region contains nine bitter taste receptor genes and seven odorant receptor-like genes. All of these genes contain a single coding exon, which Kim and co-workers (2003) then sequenced; they also sequenced the 3'-untranslated region and 300 base pairs (bp) upstream of all the genes in PTC-sensitive individuals showing linkage to chromosome 7. A detailed SNP analysis narrowed their search to a single gene, the *TAS2R28* bitter receptor gene, which the authors then designated *PTC*.

Table 1. Polymorphisms within the *PTC* gene

Position (bp)	*Position (amino acid)*	*SNP Allele*	*Amino Acid Encoded*
145	49	C or G	Pro or Ala
785	262	C or T	Ala or Val
886	296	G or A	Val or Ile

The single exon of the *PTC* gene encodes a G-protein linked receptor, 333 amino acids in length, with seven-transmembrane domains. Kim and co-workers identified three common SNPs associated with PTC sensitivity, each of which results in changes to the amino acid sequence of the PTC receptor (Table 1).

The SNPs were also shown to be inherited together in certain combinations, e.g., haplotypes (Table 2).

Table 2. SNP haplotypes of the PTC gene within the study group (named for the first letter of the amino acid present at positions 49, 262, and 296.

Haplotype	*European Freq.*	*East Asian Freq.*
PAV	49%	70%
AVI	47%	30%
AAV (from recomb. at aa 49)	*3%*	-
A later screen identified two additional haplotypes, PVI and AAI, which were found only in individuals of sub-Saharan African ancestry. The AVI nontaster haplotype was found in all populations except Southwest Native Americans (Kim *et al*., 2003).		

Although each individual SNP showed strong association with PTC taster status, certain haplotypes were even more definitively associated with taster status (Table 3).

Table 3. Genotype association with taste phenotypes (by haplotypes)

Genotype (diploid)	*Nontasters*	*Tasters*
AVI/AVI	59 (81%)	14 (19%)
AVI/AAV	11 (52%)	10 (48%)
*/PAV	4 (2%)	166 (98%)

*= PAV, AVI or AAV. No AAV homozygotes were observed in the study group. The total number of PTC genotypes observed was thus 5. The above results represent pooled numbers for two different study groups (families and unrelated individuals). The AVI/AVI nontaster phenotype was even more definitive in the unrelated study group, e.g., 21 out of 21 nontasters (Kim *et al*., 2003).

One question to explore in this project is how the AVI haplotype alters the structure and/or function of the PTC receptor, leading to a nontaster phenotype in most AVI homozygotes. A non-taster phenotype may be because (a) the PTC receptor is not present in the plasma membranes of the taste receptors in non-tasters; (b) the non-taster PTC receptor may not bind PTC with the same affinity as the taster receptor; or (c) the non-taster receptor may bind PTC but is defective in a downstream activation step. Research group members should consider these additional questions as they explore this topic: What is the normal structure and function of the PTC receptor? Where within the structure do the non-taster variant amino acids reside? What is the side chain structure of the taster (PAV) and non-taster amino acids (AVI)? How do the variant amino acids alter the structure and/or function of the protein? For answers to some of these questions, group members should read the paper by Floriano and co-workers (2006), in which they describe in-depth, three-dimensional modeling of the protein.

It should be noted that PTC taste sensitivity is not an all or nothing trait, displaying instead a broad and continuous distribution (e.g., it behaves like a quantitative trait). However, the average PTC taster scores in the Kim study were highest for the PAV/PAV homozygotes, slightly but significantly lower for the PAV heterozygotes, and lowest by far for the AVI/AVI homozygotes. The rare AVI/AAV heterozygotes had a mean PTC score slightly, but significantly, higher than the AVI/AVI homozygotes. Finally, sequencing the PTC gene from several non-human primates determined that all were homozygous for the PAV form. Thus, the AVI nontaster haplotype arose after humans diverged from the most recent common primate ancestor (Kim *et al*., 2003). In a fascinating instance of molecular convergent evolution, the PTC nontaster phenotype in chimps appears to be due to a mutation of the initiation codon of the PTC gene, such that a downstream ATG is used as the start codon for translation, resulting in a truncated protein that does not respond to PTC (Wooding *et al*., 2006).

A final, yet intriguing, question is what the mechanism was for the widespread appearance of the nontaster AVI haplotype in humans. Since bitter compounds are also often toxic, it is not at all clear what, if any, selective advantage a nontaster phenotype for bitter compounds would have had in ancestral human populations.

Student Outline

Summary of Project Protocol

On Day 1 of this project, each researcher will amplify approximately 900 bp of the coding sequence of the PTC gene, which includes all three SNPs associated with PTC sensitivity. Each researcher will prepare three cheek cell DNA samples for PCR: his/her own DNA and that of two other individuals. Each human volunteer will fill out a consent form and questionnaire, which includes doing a blind taste test using the control and PTC strips (make sure the volunteers rinse their mouths out with water if they have just eaten anything).

The success of the PCR reactions will be determined by gel electrophoresis on Day 2 of the project, and DNA from successful PCR reactions will be purified and prepared for shipping to the Biotechnology Resource Center at Cornell University for sequencing on Day 3. Investigators will then use NCBI's BLAST search engine to compare each of their sequences with those in NCBI's gene database to make sure they have in fact amplified the correct region of the human genome. More detailed sequence analyses to identify the SNPs and the corresponding PTC amino acid haplotypes for each individual will then be done using the Lasergene software suite. All members of the group will compile their individual results in a table, to assess the correlation between PTC taste sensitivity and the particular haplotypes and genotypes. A flow chart for the procedural steps to complete the project is in Figure 1.

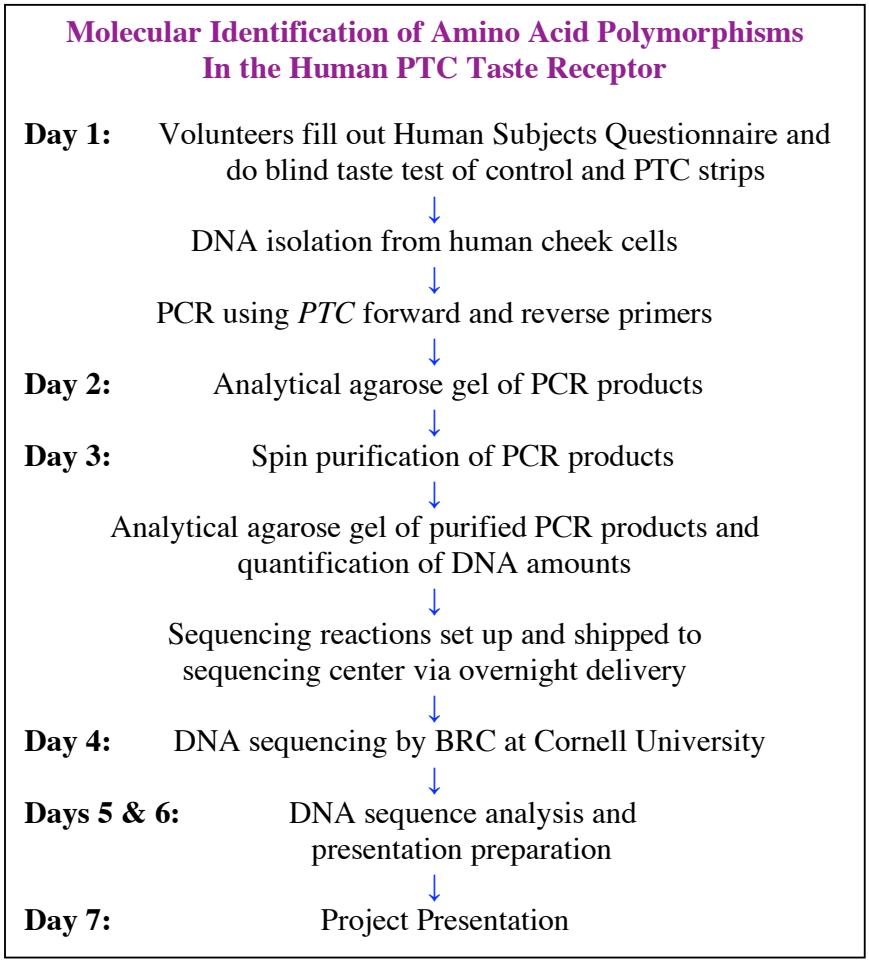

Figure 1. Flow chart of steps for sequence analyses of the human *PTC* gene and protein.

Notes for the Instructor

Bench Research Project

A Power Point file containing slides to introduce this project is available at the Amherst College Summer Teachers' Workshop Web site:
https://www.amherst.edu/academiclife/departments/Biology/summerworkshop/manual/bitter.
A PDF of a Microsoft Word file that contains the introduction in this paper, followed by a detailed protocol for the entire project – from DNA preparation to sequence analyses and project presentation - is also linked to this Web page. A list of reagents and instructions for their preparation is in Appendix III of the linked PDF, and a detailed Excel spreadsheet of materials and supplies (including vendors) is also available at the above Web site.

Dry Lab Activities

Two 'dry lab' versions of this project have been developed for instructors who do not have the time and/or laboratory resources to do DNA isolation, PCR, and DNA sequencing. In the 'dry lab' activities, the project is once again introduced using the Power Point slides, and all study participants then do a taste test using the control and PTC taster strips. The instructor then provides each study participant with either raw DNA sequence files or a pre-printed chromatogram, both of which match the PTC tasting phenotype of the subject (similar to what the study participant might have generated if the DNA had actually been sequenced). In the former case, participants work with the raw DNA sequence files in a computer lab, following the directions starting on page 12 of the detailed protocol. DNA sequence files corresponding to each of four taster phenotypes (taster, medium taster, weak taster, and nontaster) are linked to the Amherst College Teacher's Workshop Web site.

If a computer lab is not available, each study participant can instead receive a color handout of a DNA sequence chromatogram and scan through a short stretch of DNA sequence that contains the three *PTC* SNPs. Participants follow directions in Part D (#1-6) on pages 14-15 and use Table 1 and the *PTC* DNA sequence information in Appendix I of the detailed protocol for this activity. They then jump to question #12 in Part D on page 16 to explore the structural implications of the three amino acid variants.

Protein Structure Analyses

The Instructor's Power Point file also has slides, including some with figures taken from the Floriano *et al.* (2006) paper, to provide instructors with a sense of what students may discover about the structure of the PTC taste receptor and the structural effects of the three amino acid substitutions in taster variants. Students will hopefully come to a similar level of understanding/explanation in their own analyses and presentations. Instructors who have previously done a protein structure lab with their students (including computer modeling of 3-D structures) may wish to expand this part of the project to include a more detailed analysis of the Floriano *et al.* paper (2006) and the structures contained therein.

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About the Author

Dr. Julie Emerson is the Lab Coordinator for the Department of Biology at Amherst College and the Director of the HHMI Summer Teacher's Workshop. She has taught a variety of courses in molecular, cellular, developmental, and organismal biology at several institutions across the United States. At Amherst, she teaches in the Biology 181 and Biology 191 courses, in which students obtain a comprehensive introduction to many key biological concepts and underlying scientific approaches, methodologies and pedagogical approaches to learning.

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