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Mini Review

Genetics of individual differences in bitter taste perception: lessons from the *PTC* gene

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The ability or inability to taste the compound phenylthiocarbamide (PTC) is a classic inherited trait in humans and has been the subject of genetic and anthropological studies for over 70 years. This trait has also been shown to correlate with a number of dietary preferences and thus may have important implications for human health. The recent identification of the gene that underlies this phenotype has produced several surprising findings. This gene is a member of the T2R family of bitter taste receptor genes. It exists in seven different allelic forms, although only two of these, designated the major taster and major non-taster forms, exist at high frequency outside sub-Saharan Africa. The non-taster allele resides on a small chromosomal region identical by descent, indicating that non-tasters are descended from an ancient founder individual, and consistent with an origin of the non-taster allele preceding the emergence of modern humans out of Africa. The two major forms differ from each other at three amino acid positions, and both alleles have been maintained at high frequency by balancing natural selection, suggesting that the non-taster allele serves some function. We hypothesize that this function is to serve as a receptor for another, as yet unidentified toxic bitter substance. At least some of the remaining five haplotypes appear to confer intermediate sensitivity to PTC, suggesting future detailed studies of the relationships between receptor structure and taste function.

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The human sense of taste consists of five different modalities, bitter, sweet, sour, salt, and umami (the taste elicited by glutamate), that are critical for nutrition and survival. Of these, bitter perception has a particularly important role, as it protects us from ingesting naturally toxic substances which typically taste bitter. Two important questions regarding bitter taste currently exist. First, how are the wide variety of chemical structures and chemical classes all recognized as bitter, and second, what mediates interindividual differences in bitter taste?

Bitter perception generally occurs through bitter taste receptors located on the surface of taste cells of the tongue (1). These receptors are encoded by *T2R* genes that show 25–89% amino acid sequence identity between the 25 different members of this gene family. These differences presumably allow a wide variety of different chemical shapes, sizes, and functionalities to be bound by these receptors and perceived as bitter.

Psychophysical studies have showed that large individual differences in the bitterness of taste compounds exist (2). The best known example of variation in sensitivity to a bitter compound is that of phenylthiocarbamide (PTC) (Fig. 1). Virtually, all human populations studied to date display bimodality in sensitivity to PTC, such that approximately 75% of individuals worldwide perceive this compound as intensely bitter, while to others, this compound is relatively tasteless. This difference has motivated the use of PTC in many studies of taste perception in humans (3, 4), and over the past 70 years, these studies have provided many insights in human psychophysics and physiology. However, the genetic factor(s), which is responsible for this phenotypic variation,

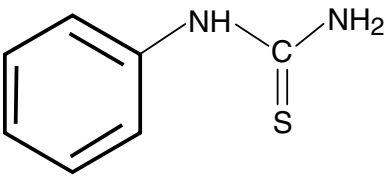

Fig. 1. Chemical structure of phenylthiocarbamide.

remained uncharacterized until recently. In this review, we focus on our recent discovery of the *PTC* gene, the developments in taste physiology and population genetics that have followed, and the implications of this work for understanding phenotypic variation in taste perception in general.

The discovery of taste blindness for PTC

Studies on the genetics of taste perception for PTC began in the early 1930s with the accidental finding by A. L. Fox that crystals of PTC tasted very bitter to some people but not to others (5, 6). This difference, which is stable over the lifetime of a given individual, was typically measured using PTC-saturated paper and recording subject's perception, either bitter or not. However, later improvements in measurement utilized a PTC dilution series and forced choice blind sorting tests to determine an individual's level of PTC sensitivity. These quantitative studies showed that between the most sensitive and least sensitive individuals, the ability to discriminate PTC in solution varied by four orders of magnitude in PTC concentration. Using a variety of testing measures, researchers have made estimates of the frequency of PTC 'tasters' and 'non-tasters' in many different populations worldwide. These studies showed that although non-tasters exist in virtually all populations (the exception being one small group of Brazilian Indians), the frequency of such individuals varies in different populations (3). The best studied population is Caucasians, where the frequency of non-tasters is estimated to be approximately 28% overall.

Mode of inheritance

Soon after the discovery of PTC sensory variation, several investigators reported that this trait is inherited, and that the non-taster phenotype was transmitted as a simple Mendelian recessive trait (7, 8). However, other studies have suggested that incomplete dominance, multiple alleles, or multiple genes fit the inheritance of the inability to taste PTC better than a simple Mendelian recessive model (3). In particular, Reddy and Rao (9) examined inheritance patterns in a sample of 100 families and suggested that PTC taste sensitivity is controlled by a major locus with incomplete dominance, as well as by a multifactorial component with significant residual heritability. Olson et al. (10) studied 120 families and produced results that supported a two-locus model in which one locus controls PTC tasting and the other locus controls a more general taste ability. Thus, it became clear that PTC taste ability is not simply Mendelian in its transmission pattern, and rather than being a dichotomous trait, it displays a broad and continuous, albeit bimodal, distribution of taste sensitivity in the population (11).

Genetic linkage analysis

Linkage evidence for PTC taste ability has a long history of conflicting findings. Chautard-Freire-Maia (12) searched for linkage using data on 22 autosomal polymorphic loci collected by Morton and his colleagues (13, 14) from a northeastern Brazilian population. This study found evidence for linkage between PTC phenotype and the KEL blood group antigen, later determined to reside on chromosome 7, in the region of band q^3. The study of Conneally et al. (15) added significant support to the evidence for linkage to KEL, with a total combined LOD score of 10.78 at $\theta = 0.045$. However, a number of other studies failed to find significant support for this linkage (16–18). These linkage studies were performed assuming recessive inheritance of the trait and assigning subject phenotype in a dichotomous fashion as a qualitative trait, either taster or non-tasters. At this point, linkage studies of PTC taste ability reached an impasse that was not overcome until the advent of polymorphic DNA markers and high-resolution genome-wide linkage scans.

The first such linkage scan was performed with the related compound propylthiouracil (PROP) in a Caucasian population (19). In this study, the phenotype was measured by using a single subjective rating of taste intensity with six categories of taste strength (20). In an important advance, these phenotypic data were treated as a quantitative trait and produced significant evidence of linkage to a locus on chromosome 5p (t-score = 3.28, $p = 0.0005$), along with a suggestion of linkage to markers on chromosome 7q31 at a distance of approximately 35 cM from KEL (t-score = 2.34, $p = 0.008$). Thus, this study, employing a quantitative trait loci (QTL) model and genome-wide marker information but a slightly different taste compound, produced quite different results.

In attempt to address this problem comprehensively, we obtained a quantitative measure of PTC-tasting ability using the modification of Harris and Kalmus method (11) in 26 Utah Center d'Etude du Polymorphsime d'Humain (CEPH) families, consisting of 267 individuals (21). With this information, we performed linkage analyses with both the recessive model based on the traditional taster and non-taster classification of the trait and QTL using the full range of PTC thresholds. These analyses showed repeatedly significant evidence for linkage to chromosome 7q35–36, consistent with some of the previous results provided by earlier studies (12, 15). In addition, the result a of quantitative trait analysis using two-locus whole-genome scan conditional on the chromosome 7 QTL identified a secondary locus on chromosome 16p. Interestingly, we observed that non-taster phenotypes in the families showing linkage to chromosome 7 were qualitatively different from the non-taster phenotype in other families not linked to chromosome 7. Non-tasters in chromosome 7-linked families typically reported no taste whatsoever from PTC solutions in the range reliably detected by tasters. Non-tasters in families showing no linkage to chromosome 7 often reported some sensation or taste other than bitter at such PTC concentrations. This implied the existence of two types of non-tasters, consistent with previous suggestions (10).

Identification of the gene underlying PTC phenotype

While many studies have supported the view that PTC sensitivity represents a Mendelian trait (7, 8), it became clear that this phenotype displays many characteristics that have hampered traditional linkage and positional cloning studies of complex traits in humans, including a continuously distributed phenotype, uncertain mode of inheritance, low LOD scores, and failures to replicate linkage. This indicated that the PTC-taste phenotype occupies an intermediate position between that of typical Mendelian disorders and fully complex traits. This suggested that identification of the gene(s) for this trait might provide a better understanding of the genetics of complex traits in general.

Understanding of bitter taste perception has been aided by many recent advances of *in vitro* studies in bitter taste perception (1, 22) and by the Human Genome Project, which has provided candidate gene information. With strong evidence for linkage to chromosome 7 using qualitative and quantitative analyses, our group focused on identifying the gene which resides at this locus (21, 23). Based on this, we hypothesized that the CEPH families that showed linkage only on chromosome 7 and nowhere else in the genome were the result of a single recessive gene. We then performed additional genotyping for high-resolution linkage and haplotype studies and undertook a detailed candidate gene search in this region. In the critical linkage interval on chromosome 7, we found a number of *T2R* bitter taste receptor genes, plus a number of other genes tentatively annotated as odorant receptor-like genes, which together were evaluated as leading candidates. Sequence analysis of these genes identified one variant in a *T2R* bitter receptor gene that demonstrated strong association with taste phenotype in chromosome 7-linked CEPH families. Further analysis with an independent group of subjects using 50 single nucleotide polymorphisms (SNPs) at an average spacing of 50 kb across the interval demonstrated strong association with phenotype and linkage disequilibrium (LD) within a 150-kb region of the interval. Within this region, 35 out of 37 unrelated non-tasters were homozygous for a conserved haplotype, largely as predicted for a recessive allele. Bioinformatics and gene-finding efforts within this region revealed only one gene, the *T2R* bitter receptor gene which originally showed strong association with the phenotype and LD in the Utah CEPH families.

The phenotype/genotype correlation

This gene, now designated as *T2R38* or *PTC*, is a member of bitter taste receptor gene family in humans (1). It consists of a single coding exon 1002 bp long, encoding a 333 amino acid, 7-transmembrane domain G-protein-coupled receptor (23). Within the gene, we identified three common SNPs, all of which result in amino acids changes in the protein. One of these variants, which substitutes an alanine for a proline at amino acid residue 49 (A49P), showed a strong association with taster status in the subjects studied.

We then characterized this gene in African, European, Asian, and Native Amerindian populations, and identified five different haplotypes specified by this gene, involving different combinations of variants at three different positions in the protein amino acids 49 (proline or alanine), 262 (alanine or valine), and 296 (valine or isoleucine). The two most common haplotypes, designated proline-alanine-valine (PAV) and alanine-valine-isoleucine (AVI), showed

a very strong association with taste status, and PAV is now referred to as the major taster haplotype, while AVI is referred to as the major non-taster haplotype. Individuals who carry two copies of AVI haplotype were largely non-tasters, whereas either one or two copies of the PAV haplotype were largely tasters. Also, a modest heterozygote effect was apparent. As a group, PAV/AVI heterozygotes had a statistically significant higher PTC taste threshold than PAV homozygotes ($p < 0.005$) and thus were slightly less sensitive to PTC. In addition to the common PAV and AVI haplotypes, a third haplotype, AAV, was observed at lower frequency. When scored on a simple dichotomous measure of PTC sensitivity, AVI/AAV heterozygotes were almost evenly divided between tasters and non-tasters. This suggests that AAV haplotype specifies a more intermediate phenotype. Overall, this study demonstrated that these haplotypes completely explain the bimodal distribution of PTC taste sensitivity, and 55–85% of the variance in PTC sensitivity, depending on the population studied.

Because the mode of inheritance of PTC-taste sensitivity has been a subject of controversy (3), we examined the evidence for additional genetic contributions to PTC linkage score. The results were largely consistent with a model of a major recessive QTL modified by either a polygenic or a single-locus residual background effect (9, 10). It also supported the view that additional genes outside of chromosome 7q contribute to this phenotype, and that one such gene might reside on the short arm of chromosome 16 (21).

Population genetics and molecular evolution

The findings with the *T2R38* gene and PTC raised an important question. Why is the frequency of the AVI non-taster allele so high? Because our sense of bitter taste is thought to protect us from ingesting toxic substances, a non-functional allele of a bitter taste receptor might be expected to be quickly eliminated from the population by natural selection. Even though PTC itself has not been found in nature, it is structurally related to a group of compounds that occur in cruciferous vegetables (e.g. cabbage, broccoli, and Brussels sprouts) and are toxic in large quantities, with the thyroid as the primary organ affected. For example, isothiocyanates and goitrin are bitter PTC-related compounds caused by hydrolysis of glucosinolates naturally present in raw cabbage (24). Variable aversions to these compounds, correlated with PTC taster status, have been implicated in the variable rates of thyroid deficiency (25).

This question has long intrigued geneticists. For example, Fisher et al. (26) tested PTC tasting in chimpanzees and found that the proportion of non-tasters is approximately the same as in humans (approximately 30%). They speculated that the existence of this phenotypic polymorphism in both humans and apes would have not occurred without natural selection, and they proposed that balancing selection has maintained both taster and non-taster alleles in the population. Such hypotheses, however, have been difficult to test without knowledge of the gene encoding this trait.

Recently, Wooding et al. (27) performed a global survey of the *PTC* gene in a sample of 165 individuals collected from African, Asian, European, and North Amerindian populations, along with apes for comparison. In addition to the three common SNPs found previously, two rare human variants were identified only in populations of African origin, one at amino acid position 80 and one at position 274. Together, these five coding SNPs specify seven different haplotypes in the worldwide population. Furthermore, this study revealed that the two major taster and non-taster haplotypes, which account for >90% of all chromosomes (23), were found at intermediate frequencies across all populations. The chimpanzee and gorilla were both homozygous at all nucleotide positions and thus carried one haplotype each, which was the PAV type, analogous to human tasters. Human and chimpanzee sequences differed by an average of 8.3 nucleotides as did human and gorilla sequences indicating the high similarity between human and the great apes.

Measurement of genetic differentiation between the continental population samples by use of F_{ST} statistics showed that this was low ($F_{ST} = 0.056$) in comparison with estimates based on other genes. Different tests of natural selection including Tajima's D demonstrated a significant deviation from neutrality because of an excess of intermediate-frequency variants when human population growth was taken into account. The high Tajima's D-value along with a low F_{ST} value supported the hypothesis that balancing natural selection has acted to maintain 'taster' and 'non-taster' alleles at the *PTC* gene in humans. The estimated divergence date of two common haplotypes was approximately 1.5 million years under the assumption that the nucleotide substitution rate in *PTC* is 10^{-9}/site/year, although this conclusion remains uncertain because of small number of mutations and the limited resource of chimpanzee data.

It now appears that balancing natural selection has maintained divergent PTC alleles in human

populations, but the mechanism by which this happens still needs to be resolved. Given the fact that the alterations present in the non-taster allele are not obvious null mutations, one possibility is that the PTC non-taster allele encodes a receptor that is fully functional for a different but as yet unknown bitter toxic substance.

Applications

Understanding of the multiple sequence variations in the *PTC* gene will allow examination of the details of the genetic basis of food preferences and the relationship between bitter taste sensitivities and possible health outcomes.

Identification of the *PTC* gene and studies of its molecular evolution have revealed a number of different aspects that must be considered together in future studies of the genes responsible for inter-individuals' difference in bitter taste sensitivity. First, unlike PTC, PROP, and related compounds, the distribution of taste thresholds for other bitter compounds typically shows unimodal distribution. Second, although natural selection has acted to maintain both taster and non-taster alleles at the *PTC* locus, it is not clear whether this phenomenon has occurred for other bitter taste receptor genes. To understand this in a better way, we will need to examine variation in the entire T2R gene family across populations, which will help human history in bitter taste perception and enable creation of comprehensive functional repertoires of each gene for studies of phenotype/genotype correlations.

On a larger scale, the *PTC* gene may be illustrative of ancient genetic variation that has been proposed to underlie common disease in modern populations. The PTC non-taster allele is common, both because it is very old and because it appears to confer selective advantage, at least in the heterozygote state. This supports the 'common disease-common variant' hypothesis and suggests that greater study of relatively older African populations could reap important benefits in the search for variation that underlies common disease.

Finally, PTC presents a unique opportunity in the field of bitter taste transduction. Having a known gene with a strong effect on phenotype *in vivo* provides many opportunities for studies of taste physiology, biochemical function, and molecular structure elucidation in the human sense of taste.

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