Physiology & Behavior 128 (2014) 148–154

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Exploring associations between taste perception, oral anatomy and polymorphisms in the carbonic anhydrase (gustin) gene *CA6*

Emma L. Feeney, John E. Hayes *

Sensory Evaluation Center, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA
Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA

HIGHLIGHTS

- 246 subjects rated multiple bitter and salty stimuli on a gLMS.
- Polymorphisms (SNPs) within the CA6 gene were examined.
- Number of fungiform papilla (FP) were quantified.
- Several SNPs associated with saltiness, but not bitterness.
- There was no association with CA6 SNPs and FP number.

ARTICLE INFO

Article history:
Received 9 October 2013
Accepted 4 February 2014
Available online 15 February 2014

Keywords:
Salt perception
Bitter perception
Carbonic anhydrase
Polymorphisms
Fungiform papillae

ABSTRACT

Recent reports suggest that polymorphisms in the carbonic anhydrase gene *CA6* (also known as gustin) may explain additional variation in the bitterness of 6-*n*-propylthiouracil beyond that explained by variation in the bitter receptor gene *TAS2R38*. *CA6* (gustin) has been implicated in taste bud function and salivary buffer capacity. In the present study we examined associations between polymorphisms in the *CA6* gene with salt and bitter taste perception, and oral anatomy. 243 subjects (146 female) aged 18–45 rated the intensity of five concentrations of 6-n-propylthiouracil and NaCl on a generalized Labeled Magnitude Scale (gLMS) in duplicate and one concentration of potassium chloride (KCl). Using salivary DNA, we examined 12 SNPs within *CA6* in relation to taste intensity and number of fungiform papillae. We observed no difference in bitter taste perception from 6-*n*-propylthiouracil (PROP) or from potassium chloride for any of the SNPs examined. Perceived saltiness of NaCl on the other hand was significantly associated with a number of *CA6* polymorphisms, and particularly rs3737665. Nonetheless, FP density did not vary between alleles of rs3737665, nor with any of the other *CA6* SNPs. Also, we fail to find any evidence that CA6 effects on taste perception are due to differences in fungiform papilla number. Additional work is needed to confirm whether variations within the CA6 gene may be responsible for differences in salt taste perception.

1. Introduction

Taste perception has long been known to vary across individuals. Since the discovery of 'taste blindness' to phenylthiocarbamide (PTC) in the early 1930s [1,2], substantial research has been carried out to understand the biological basis of individual differences in taste perception. The perception of the bitter-tasting compounds PTC and 6-*n*-propylthiouracil (PROP) have received particular attention as they are members of a specific class of thiourea-containing compounds that also includes naturally occurring compounds found in brassica vegetables [3,4]. Since glucosinolates in Brassica vegetables are hydrolyzed to isothiocyanantes, molecules with recognized beneficial effects (e.g. [5]), the juxtaposition of aversive taste sensations and lower vegetable intake (e.g. [6,7]) with intake of beneficial phytonutrients (e.g. [8]) has attracted substantial interest. However, only in the last decade have we been able to examine the molecular genetics underlying this dimorphism. The *TAS2R38* gene (*née PTC*; HGNC:9584) was identified in 2003 [9], and was found to encode a receptor, hT2R38, which responds to PTC and PROP in vitro and in vivo [10,11]. Because polymorphisms in *TAS2R38* associate with differences in taste perception [10–12] and vegetable intake [13], this genetic variation may have broader impact on food choice and nutritional status [14,15], although not all data support this view [16,17].

Subsequent work on *TAS2R38* haplotypes and taste perception indicated other additional unknown genetic factors might also be involved

* Corresponding author at: Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA. Tel.: +1 814 863 7129.
E-mail address: jeh40@psu.edu (J.E. Hayes).

http://dx.doi.org/10.1016/j.physbeh.2014.02.013
0031-9384/

in PROP bitterness perception [11,12,18,19], potentially located on chromosome 16 [20]. Differences in the number of fungiform papillae (FP) are often discussed as being involved in PROP perception, as FP density is thought to be a rough indicator of taste nerve innervation [21], and indeed, correlations between the number of FP and perceived bitterness [22,23] and sweetness [24] have been reported. Further, number of FP reportedly correlates with PROP taste intensity independently of *TAS2R38*, as number of FP does not differ with diplotype [12], suggesting FP number may be an anatomical marker of overall taste intensity. However, when the link between PROP and FP was explored within genetically homogenous individuals, the expected relationship between PROP and FP was absent in the *TAS2R38* heterozygotes, demonstrating that the association between FP and taste perception is not straightforward. Moreover, not all reports support the finding that number of FP is directly correlated with PROP: a recent epidemiological study found no association between PROP intensity and FP number [25]. Indeed, it has been suggested that FP number is a more accurate predictor of taste intensity perception in small areas of the anterior tongue than for whole-mouth stimulation [26].

The term 'supertaster' was first coined by Linda Bartoshuk following observations in her laboratory that PROP tasters (defined via threshold) were more varied in their perception than nontasters [27]. Using suprathreshold methods, they found that descriptions of PROP from 'tasters' ranged from mildly to intensely bitter. Traditionally, PTC/PROP tasters had been separated from nontasters using detection thresholds, or response to an antimodal concentration (see [28]). This separation agreed with the prevailing theory at the time; i.e. that the ability to taste thiourea compounds at low concentrations was a simple Mendelian-inherited dominant trait, with *T* indicating the taster allele and *t* indicating the nontaster allele. Thus, *Tt* and *TT* individuals would phenotypically be tasters, and tt individuals nontasters (although other modes of inheritance were occasionally suggested (cf. [29–31]; see [32] for a detailed review).

In 1994, Bartoshuk, Duffy and Miller published the first peer reviewed paper on supertasting, subdividing tasters into 'supertasters' and 'medium tasters' via multiple PROP and sodium chloride solutions as whole-mouth stimuli, which were rated for intensity using magnitude estimation [22]. They speculated that 'supertasters', those reporting intense bitterness from PROP, might be homozygous dominant (e.g. *TT*) with 'medium tasters' being heterozygous (e.g. *Tt*) [22], although molecular data later disproved this (e.g. [11,12]). The concept of supertasting has evolved over time, and a variety of phenotyping methods now exist to determine who is or is not a supertaster (see [33] for a review). In the original 1994 paper, a ratio of PROP intensity to salt intensity was used to categorize individuals: a ratio below 0.8 were defined as nontasters, and above 1.2 were considered supertasters [22]. Using similar logic, a graphical variant of this method uses three concentrations of salt and PROP solutions [34]. In the graphical method, the psychophysical function is plotted for each individual, and they are categorized into super, medium and nontasters depending on whether the PROP ratings are higher, the same, or lower than those for salt, respectively [34]. This can be reduced to a one solution test, which still shows high test–retest reliability [34]. It was later demonstrated that using sound rather than NaCl as a reference against which to normalize the PROP ratings resulted in greater effect sizes [35], since the intensity of a wide range of taste stimuli, including salt, increase with increased PROP perception [35–37]. Given this limitation in salt-based classification methods, other researchers have normalized PROP ratings to other modalities like tones [19] or weights [38] or remembered sensations like the brightness of the sun [39] prior to classification, while other reports use raw (unnormalized) PROP ratings and classify individuals either on the basis of a priori cutoffs [6,40] or the observed distribution in the data [41,42]. Notably, both the observed distribution method, and the a-priori cut-off method are typically based on the early assumption that a given population should have a 25/50/25 split, with the lower quarter being nontasters, the upper quarter being supertasters and the remainder being medium tasters. Simple Mendelian genetics would dictate that the population should have the proportions 25% *tt*, 50% *Tt*, and 25% *TT*. However, this assumption may not be valid, given newer data.

Other work suggests PROP bitterness is a continuous variable and should be treated as such (e.g. [21], although traditional trimodal classification schemes may reflect the underlying distribution of larger populations [43]. Most work uses liquid stimuli, although PROP has also been delivered via filter paper discs [44] or dissolving strips [45] placed on the tongue; such methods are particularly useful for conducting large-scale studies outside of the laboratory environment (e.g. [17,46].

In addition to variability from different classification schemes, choice of scaling methods may hinder comparisons across research groups and populations. Although the field has largely settled on the general Labeled Magnitude Scale (gLMS) [47] or its predecessor, the Labeled Magnitude Scale (LMS)[48], use of other psychophysical scales complicates interpretation of earlier reports (e.g. [49–52].

Regardless of the classification method, it has often been shown that PROP bitterness perception correlates with greater intensity ratings from other taste stimuli. However, Lim et al. [53], state that other taste stimuli may be better markers of general taste ability, due to the bimodal nature of PROP taste perception. Using NaCl, sucrose, citric acid and quinine as the taste stimuli, they found that while the four taste stimuli correlated to each other, PROP correlated only to the bitterness of quinine. Rather than the typical suggestion that number of taste buds or FP density could explain covariation between PROP and other tastants, Lim and colleagues suggested that correlated intensities might be due to a central gain mechanism; whether this mechanism might have a simple genetic correlate is unknown.

The role of taste bud density in generalized supertasting (hypergeusia) [33] has been revisited recently, with a report which suggested PROP taste intensity also associates variations in the *CA6* (gustin) gene [54]. The *CA6* gene encodes the carbonic anhydrase VI protein, an enzyme that catalyzes the hydration of carbon hydroxide in saliva, [55] and is thought to have an important role in taste bud function. A SNP in CA6, rs2274327 (Thr55Arg) results in different variations, which have been implicated in salivary buffer capacity; in individuals with the highest buffer capacity, those with two thymine nucleotides (i.e. TT allele carriers) were significantly lower than expected by chance [55]. A range of other SNPs within *CA6* were examined in that report, but rs2274327 appeared to be the only functional SNP, at least with regard to buffer capacity.

Padiglia and colleagues [14] examined the rs2274333 SNP within *CA6* and observed that 'A' (adenine) alleles were more frequent in supertasters (as defined by a graphical PROP:salt ratio). The authors speculated the differences in taste intensity might have been due to varying FP density, although they did not measure FP in their report. Therefore, an open question remains as to whether SNPs within the *CA6* gene are related to taste intensity for other taste stimuli. Also, it is unknown whether number of FP varies with polymorphisms within *CA6*. Thus, the goals of the present study were to: a) examine putatively functional SNPs in the *CA6* gene as predictors of variation in suprathreshold taste intensity for salty and bitter tastants, and b) assess potential relationships between *CA6* SNPs and number of fungiform papillae.

2. Materials and methods

2.1. Participants

243 reportedly healthy participants (146 women), aged 18–45 were recruited from the Pennsylvania State University campus and surrounding area. Written, informed consent was obtained from each participant and participants were reimbursed for their time. Individual test sessions with the participant and experimenter took approximately 60 min to complete, of which 5–10 min was spent photographing the tongue.