HHS Public Access

Author manuscript

Nutr Res. Author manuscript; available in PMC 2016 December 01.

Published in final edited form as:

Nutr Res. 2015 December ; 35(12): 1122–1125. doi:10.1016/j.nutres.2015.09.011.

A potential trigger for pine mouth: a case of a homozygous PTC taster

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Abstract

Pine mouth, also known as Pine Nut Syndrome (PNS), is an uncommon dysgeusia that generally begins 12–48 hours after consuming pine nuts. It is characterized by a bitter metallic taste, usually amplified by the consumption of other foods, which lasts 2–4 weeks. Recent findings have correlated this disorder with the consumption of nuts of the species *Pinus armandii*, but no potential triggers or common underlying medical causes have been identified in individuals affected by this syndrome. We report a 23-year-old patient affected by pine mouth that also underwent a PTC taste test and was found to be a taster for this compound. *TAS2R38* genotyping demonstrated this subject was a homozygous carrier of the PAV taster haplotype. We therefore hypothesize that homozygous PTC taster status may be a potential contributor for pine mouth events. Although based on a single observation, this research suggests a connection between genetically determined bitter taste perception and the occurrence of pine nut dysgeusia events.

Keywords

Dysgeusia; Pine nuts; PTC; *TAS2R38*; Taste disturbance

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The authors report no conflicts of interest or financial relationships to disclose. The authors alone are responsible for the content and writing of the paper.

1. Introduction

In the past five years, studies have reported that thousands of cases of individuals in Europe and the USA have experienced a persistent bitter taste reaction to pine nuts [1–3]. The classical symptoms of this disorder, also known as pine nut syndrome (PNS), are a delayed and constant bitter or metallic taste, occurring one to three days after the ingestion of pine nuts and lasting up to several weeks [4]. In addition, occasional cases of diarrhea, headache and nausea have been reported to be associated with this syndrome [1]. Only a few studies of PNS have been reported [3–5], and one study has described the symptoms of pine nut-related dysgeusia in a total of six subjects [6]. A single species of pine nuts (*Pinus armandii*) has previously been associated with this syndrome [1,7,8], although recent studies have reported taste disturbances from samples containing nuts from a mixture of different *Pinus* species [2,9,10]. However, the syndrome occurs in only a small fraction of individuals who consume pine nuts, and efforts to identify a common trigger or medical cause responsible for the occurrence of PNS have not been successful [4]. The rate of resolution of PNS symptoms has been noted to be similar to the turnover rate of taste cells of the tongue, and PNS has been hypothesized to act through taste perception pathways [4]. A widespread genetic difference in taste perception is specified by the *TAS2R38* gene, which encodes a bitter taste receptor that occurs in two common forms, designated PTC taster and non-taster [11]. Taster individuals have been shown to be more sensitive to bitter taste, and genetic responsiveness to PTC may affect eating habits and food choices [12,13]. For this reason, we completely sequenced the *TAS2R38* gene in a 23-year-old women affected by pine mouth, in order to investigate potential correlations between *TAS2R38* haplotypes and the occurrence of this syndrome. In addition, a supra-threshold PTC solution was used to test the taster status tasted in this subject. The objective of our research was to investigate the hypothesis that PTC taster status may be a potential trigger for pine mouth events.

2. Methods and materials

2.1 Case presentation

A white 23-year-old American female participated in a PTC taste perception study and subsequently reported a persistent metallic/bitter taste in her mouth which began approximately 24 hours after eating a salad containing pine nuts from *Pinus sibirica* species. These symptoms worsened during the following four days and progressively improved without medication. Other symptoms (i.e. nausea, diarrhea and headache) were not reported by the subject. The patient was a non-smoker and did not have any history of medication, infections or surgery associated with dysgeusia, or any neurologic conditions.

2.2 Experimental protocol

Supra-threshold measurements of both PTC and PROP have been shown to provide strong correlations between TAS2R38 genotypes and phenotypes [14,15,16]. A single PTC concentration of 256 uM [11] was therefore chosen as the best single discriminant between taster and non-taster status. The subject was asked to hold this solution in her mouth for 10s and rate its bitterness on a Labeled Magnitude Scale (LMS) (Fig.1) [17].

2.3 DNA collection, extraction and genotyping

A saliva sample was collected using an Oragene collection kit (Genotek Inc., Kanata, Ontario, Canada) and genomic DNA was purified according to the manufacturer's protocol [18]. *TAS2R38*, the gene encoding the bitter taste receptor responsible for PTC perception, was completely sequenced using dideoxy Sanger sequencing [19], with a dedicated set of primers modified from Kim et al. [11] (Table 1). DNA chromatograms were analyzed with the Lasergene suite (DNASTAR, Madison, Wisconsin) [20].

2.4 Ethical standards

This study has been approved by the NIH CNS/Blue Panel IRB (NIH protocol 01-DC-0230) and all procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. Written informed consent form was obtained from the patient included in this study.

3. Results

Physical examination performed on the subject reported no significant findings: ears, nose and oral cavity were clear. There were no neck adenopathy or masses and no dermal lesions. Regarding the PTC tasting, the subject defined the perceived bitterness to be “between strong and very strong”, marking a value of 40 on the LMS scale, consistent with her being a PTC taster. We therefore explored and confirmed the taster status of this individual at the genetic level: she was found to carry a proline, alanine and alanine at amino acid positions 49, 262 and 295, respectively. This demonstrated that she is homozygous for the PAV (PTC-taster) haplotype (Figure 2).

4. Discussion

A recent survey of pine mouth events in U.S. has shown that the delayed dysgeusia following pine-nut ingestion is an emerging phenomenon particularly related to the ingestion of nuts from *Pinus armandii* [4]. Removal of these nuts in food has been associated with a lower number of PNS events [4]. The specific agent responsible for these symptoms, however, remains unknown and researchers have found no clear association between PNS and underlying medical conditions, age, or tobacco use. Here, we hypothesize that the PTC taster status, encoded by the *TAS2R38* bitter receptor gene, could be a common feature of people who experience PNS. Multiple anecdotal reports have noted the co-occurrence of PNS and the ability to taste PTC [21]. In addition to these, we have here shown that the homozygous taster *TAS2R38* genotype, which is associated with higher sensitivity to PTC, PROP, and many other compounds [11, 22, 23], is associated with PNS in a 23-years-old female. This subject was affected by PNS after ingesting pine nuts from *Pinus sibirica*, which have previously not been associated with PNS [1, 7,8]. However, recent reports of taste disturbances from samples containing nuts from a mixture of *Pinus sibirica* and *Pinus armandii* have been reported [2,9,10]. As a study based on a single individual, is it intended to be hypothesis-generating research with limited generalizability. Confirmation of this hypothesis in a larger number of individuals is needed, possibly by introducing a simple

PTC/PROP taste test in toxicological investigations and clinical evaluation of PNS. At this time, the only current available intervention is avoidance of recurrent exposure.

Acknowledgment

DR, CVW and DD were supported by the National Institute on Deafness and Other Communication Disorders Intramural grant numbers Z1A-000046-15 and ZIA-DC-000075.

Abbreviations

PTC	phenylthiocarbamide
PROP	propylthiouracil
PNS	pine nut syndrome
LMS	labeled magnitude scale
DNA	deoxyribonucleic acid
PAV	proline-alanine-valine

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Research highlights

- Pine nut syndrome is an uncommon dysgeusia characterized by a bitter metallic taste
- No potential triggers or common underlying medical causes have been identified so far
- We examined one subject affected by pine nut syndrome who also who also underwent a PTC taste test and *TAS2R38* sequencing
- We provide a new hypothesis for the occurrence of pine nut syndrome events, which implies a connection between TAS2R38 taster haplotype and the studied syndrome

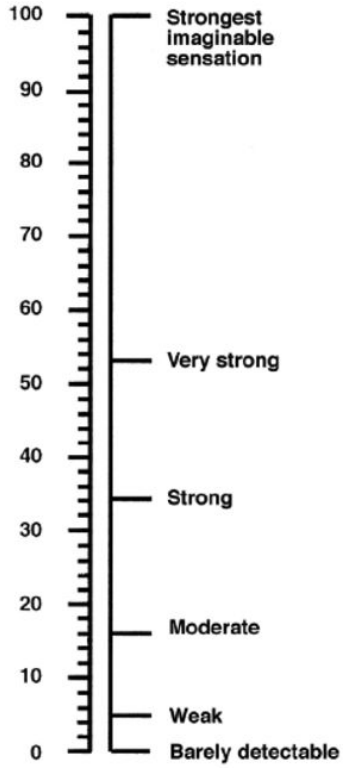

Figure 1.
The Labeled Magnitude Scale (LMS) used to evaluate PTC bitterness perception.

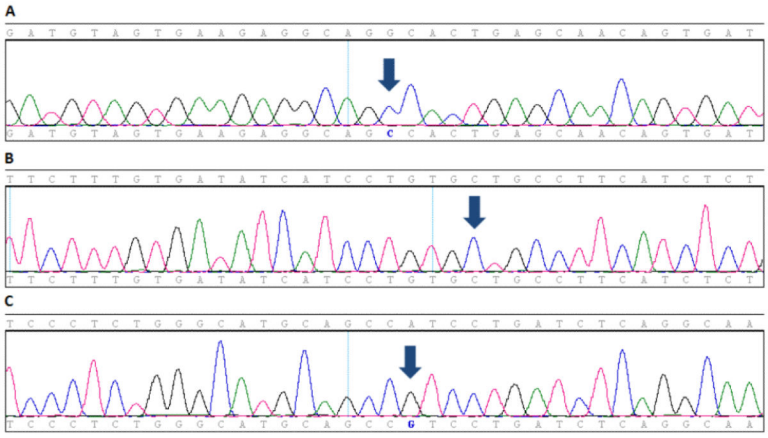

Figure 2.

Single Nucleotide Polymorphisms (SNPs) identified at amino acid positions 49 (A), 262 (B) and 295 (C) of *TAS2R38* gene. The sequence above the chromatogram indicates the reference sequence, the presence/absence of the SNP derived allele is indicated with an arrow.

Table 1

PCR and sequencing primers used to amplify and sequence *TAS2R38* gene.

Primer Name	Direction	Sequence	Reaction
TAS2R38F	Forward	AGATGGGCATGCAAAACTGG	PCR, Sequencing
TAS2R38R	Reverse	ACTCACAGGCGTATTAATGAAGA	PCR, Sequencing
TAS2R38F1	Forward	TCACACCTTCCTGATCTGCT	Sequencing
TAS2R38R1	Reverse	AGGCTGGGGTCACGAGAG	Sequencing