RESEARCH ARTICLE

Personalized expression of bitter ‘taste’ receptors in human skin

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Abstract

The integumentary (i.e., skin) and gustatory systems both function to protect the human body and are a first point of contact with poisons and pathogens. These systems may share a similar protective mechanism because, as we show here, both human taste and skin cells express mRNA for bitter ‘taste’ receptors (TAS2Rs). We used gene-specific methods to measure mRNA from all known bitter receptor genes in adult human skin from freshly biopsied samples and from samples collected at autopsy from the Genotype-Tissue Expression project. Human skin expressed some but not all TAS2Rs, and for those that were expressed, the relative amounts differed markedly among individuals. For some TAS2Rs, mRNA abundance was related to presumed sun exposure based on the location from which the skin sample was collected (TAS2R14, TAS2R30, TAS2R42, and TAS2R60), sex (TAS2R3, TAS2R4, TAS2R8, TAS2R9, TAS2R14, and TAS2R60), and age (TAS2R5), although these effects were not large. These findings contribute to our understanding of extraoral expression of chemosensory receptors.

Introduction

Humans have at least five widely accepted types of taste receptors: salty, sour, sweet, bitter, and umami. The bitter receptors, called taste receptor type 2 (TAS2R), are G protein-coupled receptors that protect humans from ingesting toxins [1]. In the gustatory pathway when bitter compounds bind to a TAS2R protein on a taste cell, a conformational change of the protein elicits a signaling cascade. This indirectly induces the release of intracellular calcium, which leads to depolarization and neurotransmitter release, thereby activating sensory neurons that send signals to the central nervous system for bitter perception [2]. Humans have 25 bitter receptors, the TAS2R proteins, that are encoded by the TAS2R genes located on chromosomes 5, 7, and 12 (Fig 1).

Recently, scientists have identified bitter receptors in locations of the body other than the taste cells. This expression and activation of extragustatory TAS2Rs will not lead to taste perception, but instead will elicit distinct cell-type-specific physiological responses. The results of several studies have demonstrated that the extraoral expression of TAS2Rs is involved in or
regulate important biological processes germane to the nature of the tissue in which they reside. Bitter receptors have been implicated in the relaxation of smooth muscle, vasoconstriction, gut motility, bronchodilation, nutrient sensing, insulin release, and the release of the antimicrobial peptide, β-defensin [3–7]. As an example, studies performed by Lee et al. demonstrated that susceptibility to upper respiratory infection depends on an inborn genotype within one of these bitter receptor genes (TAS2R38). Gram-negative bacteria secrete a quorum-sensing molecule that is an agonist of the TAS2R38 receptor. People with non-functional alleles of this receptor are more susceptible to sinonasal infection because of impairments in this bactericidal pathway [8]. The broader implications of this result are that bitter receptors expressed in extraoral areas may be involved in innate immunity.

Building on this observation, we conducted a study to assess the gene expression patterns of all 25 TAS2R genes in skin, since it is a barrier organ and a first line of defense against invading pathogens, presenting both innate and adaptive immune functions. In addition, at least one cell type in human skin (keratinocytes) expresses olfactory receptors, which are similar to bitter taste receptors [9]. Other investigators have measured mRNA expression of a few TAS2R genes in skin with conflicting results, perhaps owing to lack of appropriate controls against genomic DNA contamination [10, 11]. Here, we combine results from a smaller biopsy study using quantitative PCR (qPCR) and appropriate controls with a larger autopsy study using an RNA-seq method to get a more complete understanding of TAS2R mRNA expression patterns in human skin.

Results

Sample integrity

RNA and DNA were extracted from 15 whole skin samples provided by the University of Pennsylvania Department of Dermatology (Tables 1 and 2) and from one fungiform taste papilla (FP) biopsy obtained from a separate donor as a representative of taste tissue. One sample (004) did not produce viable RNA (RNA integrity number equivalents = 1.0) and was eliminated from the study. Using the remaining RNA samples, cDNA was synthesized and tested for the presence of unwanted genomic DNA using the Abelson 1 (ABL1) gene [12]. This is a necessary step since the TAS2R protein-coding sequences are within single exons, and TAS2R primers cannot be designed to differentiate between genomic DNA and cDNA. Based on the results, three of the samples (005, 006, and 007) were unlikely to contain cDNA because they did not express this gene, and two (009 and 014) had residual genomic DNA after a second DNase treatment (S1 Fig). These five samples were eliminated from the study.

Samples obtained from Penn Dermatology after Mohs surgery vary in size because the procedure requires surgeons to continue removing tissue until all cancerous cells are gone and only healthy tissue remains. The depth of the surgery therefore varies by individual. The samples obtained in this study consist of healthy skin that was removed to properly close the wound at the end of the procedure. Thus, each biopsy sample is unique [13]. To characterize the skin layers and cell types represented in the biopsy samples from Penn Dermatology, qPCR was performed for seven skin-layer- and cell-type-specific markers, standardized to GAPDH. As expected, biopsy samples differed in the relative abundance of cell-layer markers (Fig 2)[14, 15].

PCR amplification

To investigate whether bitter taste receptor mRNA is expressed in human skin, PCR experiments were performed with two technical replicates for each of the 25 TAS2R genes (S2–S26 Figs), which were compared against two positive controls: (a) genomic DNA from skin and (b)
fungiform papillae cDNA. Of the 25 TAS2R genes, only three showed no expression (TAS2R1, 7, and 8), 19 showed variable expression (TAS2R3, 4, 5, 9, 13, 14, 16, 20, 31, 38–43, 45, 46, 50,

**Table 1.** Penn Dermatology subject characteristics.

| Sample No. | Age | Gender | Site              |
|------------|-----|--------|-------------------|
| 001        | 64  | F      | Face              |
| 002        | 81  | M      | Cheek             |
| 003        | 52  | M      | Scalp             |
| 004\*      | 75  | M      | Neck              |
| 005\*      | 62  | M      | Left temple       |
| 006\*      | 63  | F      | Right cheek       |
| 007\*      | 46  | M      | Left temple       |
| 008        | 70  | M      | Right cheek       |
| 009\*      | 54  | M      | Nose              |
| 010        | 87  | M      | Leg               |
| 011        | 58  | F      | Left posterior thigh |
| 012        | 56  | M      | Right cheek       |
| 013        | 52  | M      | Right leg         |
| 014\*      | 55  | F      | Left supraclavicular |
| 015        | 55  | M      | Eyebrow           |

Individual information for skin samples obtained from Penn Dermatology.

*Samples that did not pass our sample integrity tests.
and 60), and three showed universal expression (TAS2R10, 19, and 30) (Fig 3). The genomic DNA positive controls were amplified in every case; however, there was variability in TAS2R expression in the FP, suggesting that TAS2Rs are expressed at low levels even in taste tissue. This low abundance may explain the variability of expression between technical replicates, as shown in S2–S26 Figs and summarized by the yellow cells in Fig 3. PCR experiments were also performed for GNAT3, a gene encoding for the α-subunit of the taste-associated G protein gustducin, and keratin 10 (KRT10), a positive epithelial marker (Fig 3 and S27 and S28 Figs). GNAT3 was detected in taste tissue, as expected, and in four skin samples (002, 003, 008, and 015), perhaps suggesting some similarity between the pathway elicited in skin and the initial steps of the gustatory pathway. As anticipated, KRT10 was detected in FP and all skin samples. All primers are listed in Table 3.

### Quantitative PCR analysis

To quantify mRNA abundance, qPCR was performed on each of the 25 TAS2R genes standardized to the housekeeping gene GAPDH (Fig 4). The results show variable expression of the TAS2R genes across samples, which was expected based on the results of the PCR amplification experiments. The taste tissue sample showed variable expression across receptor type. We also confirmed some expression of GNAT3 in samples using qPCR standardized to the housekeeping gene GAPDH (Fig 4).

Taste-related genes are minimally expressed even in taste tissue, and our agarose gel PCR and qPCR results do not correlate exactly as expected, as is the case when expression of mRNA is near the level of detection [16]. It is possible that individual variation seen here may be due in some part to technical aspects of the biopsy procedure. Despite these limitations, these results suggested that a study of TAS2R mRNA expression with a larger sample size was warranted. To do so, we turned to a large and publicly available RNA-seq data set.

### GTEx data analysis

After appropriate approvals, we obtained RNA-seq expression data from the Genotype-Tissue Expression project (GTEx; #12732: Bitter receptor gene expression: patterns across tissues). The data were measured at the gene level in RPKM units (reads per kilobase of transcript per million mapped reads) and we extracted the expression data for 25 bitter receptor genes. The

| Characteristic | Group | N subjects Penn Dermatology (N = 9) | N subjects GTEx (N = 914) |
|---------------|-------|-----------------------------------|--------------------------|
| Age           | 20–29 | 0                                 | 68                       |
|               | 30–39 | 0                                 | 70                       |
|               | 40–49 | 0                                 | 150                      |
|               | 50–59 | 5                                 | 300                      |
|               | 60+   | 4                                 | 326                      |
| Sex           | F     | 2                                 | 311                      |
|               | M     | 7                                 | 603                      |
| Sun Exposure  | Yes   | 9                                 | 508                      |
|               | No    | 0                                 | 406                      |

Summary of demographics of nine viable skin biopsies obtained from Penn Dermatology and of 914 skin biopsies from the GTEx data set. All samples from Penn Dermatology were presumed to be sun-exposed based on the physical location of the sample, e.g., cheek; the GTEx consortium identified the samples from the lower leg as sun-exposed whereas those from the skin of the suprapubic region are identified as not sun-exposed. However, we caution that skin location is a proxy measure of long-term sun-exposure and may not be accurate in all cases.

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data analyzed consisted of 914 skin samples that varied in location (presumed to be sun-exposed from lower leg or not-sun-exposed from suprapubic region), sex, and age (Tables 2 and 4). This data set was used because RNA-seq provides more accurate detection of low-abundance transcripts and because it provided a large sample size of tissue collected following a standard procedure. There was heterogeneity of variance between the TAS2R genes, but the most highly expressed bitter receptor genes were TAS2R5, 14, 20, and 4 (Fig 5). For statistical analysis of sun-exposure, we considered only subjects who had donated both sun-exposed and not sun-exposed tissue (n = 299) and performed Kruskal-Wallis tests to detect differences in the distribution of gene expression levels based on sun-exposure. Results based on tissue type indicated significantly lower expression levels in sun-exposed skin for TAS2R14 (Median diff. = 0.084,
< 0.05), TAS2R30 (Median diff. = 0.009, p < 0.01), and TAS2R42 (Median diff = 0, p < 0.05), but significantly higher expression levels in sun-exposed skin for TAS2R60 (Median diff = 0.046, p < 0.0001) (Fig 6 and S1 Table). We also observed a small sex difference in mRNA expression. In skin from the suprapubic area, females’ expression was significantly higher for TAS2R3 (Median diff. = 0.034, p < 0.01), TAS2R4 (Median diff. = 0.126, p < 0.01), and TAS2R8 (Median diff = 0, p < 0.05) (Fig 7, S2 Table). In skin from the lower leg, females’ expression was significantly lower for TAS2R3 (Median diff. = 0.023, p < 0.05), TAS2R9 (Median diff = 0, p < 0.01), and TAS2R14 (Median diff = 0.080, p < 0.01) (Fig 7, S3 Table). Finally, there was a positive correlation between increasing age and expression of TAS2R5 gene but only in not-sun-exposed skin (p = 0.001) (Fig 8).

Discussion

Previous studies have shown bitter taste receptor expression in many tissues, including the airway, gastrointestinal tract, and testes [2]. Here, we provide a comprehensive analysis of bitter taste receptor expression in skin using two types of skin samples and three methods of analysis. We show a large-scale assessment of expression of all 25 TAS2R genes in adult human skin. By compounding our data, it was discovered that bitter receptors can be detected in the skin, though not ubiquitously. The pattern of results suggests an association between TAS2R expression and chromosomal location. For instance, there is no expression of the TAS2R gene on chromosome 5 and little to no expression of the first few TAS2Rs on chromosome 12. We found that some bitter receptors are not expressed at all, some are variably expressed among people, and some are expressed in almost all skin samples we tested. Variability in more highly expressed receptors is correlated with skin location (presumed-sun-exposed vs. non-exposed), sex, and age. Expression of the taste-related gene GNAT3 suggests that these receptors are functional in the skin and that the pathway may be G protein–dependent. Although we may speculate on the significance of these findings, we are not yet able to determine causation between the factors stated above and expression level. We must further clarify that sun
Table 3. Primer sequences.

| Primer | Sequences (5’-3’) | DNA (bp) | RNA (bp) |
|--------|-------------------|----------|----------|
| TAS2R1 | F: TGTGGTGATGCACTCTG | 813      | 813      |
|        | R: CAGGCTCAGTGTCGGCG |          |          |
| TAS2R3 | F: ACACATATCAGGAAATATG | 575      | 575      |
|        | R: TTGCCATCCTGCTTGGATT |          |          |
| TAS2R4 | F: TACAGTGTCATTGCCAATACTTGG | 749      | 749      |
|        | R: CAGGGAGGAGGAGGAGG |          |          |
| TAS2R5 | F: TGTCCTCATATAACCTCTATCC | 667      | 667      |
|        | R: CTGCCATCAGGTCGCC |          |          |
| TAS2R7 | F: TTTTTTATTGGTCTATATCCAGTGTCATG | 658      | 658      |
|        | R: GGAATATGACTTGAGGGGTAGAATAGG |          |          |
| TAS2R8 | F: CAATTTAGTTATCGCCAGAATTTGTTTGTACC | 723      | 723      |
|        | R: TTATTTTACATTTAAATGATGATGACCCAAGG |          |          |
| TAS2R9 | F: TGAATTGACCATAGGATGGTTGG | 807      | 807      |
|        | R: GATATAATTATATCCAGGTATGG |          |          |
| TAS2R10| F: GACTATTGTCACTGATG | 783      | 783      |
|        | R: AAAGAGCTCCTTTCATGG |          |          |
| TAS2R13| F: GGTCAGATGAGAGGAGCCTCCT | 742      | 742      |
|        | R: ATACAGAGAGAACGCTGTCGAAG |          |          |
| TAS2R14| F: GCTTCTGCAATCTCCTGAGAT | 796      | 796      |
|        | R: CTCCTAAATCTCCTGACCTGAGG |          |          |
| TAS2R16| F: CCTGGAATTTTTTTTAATACCTTTACATCTGCTG | 419      | 419      |
|        | R: GAAGCGCGTTCTCCTACGCTT |          |          |
| TAS2R19| F: GGTCTACTGTGTCGCATGTTATAC | 606      | 606      |
|        | R: TTTGCCTCCTGCTCTGCTCATAG |          |          |
| TAS2R20| F: GCACTGAATAATTCTATGGCCTG | 770      | 770      |
|        | R: TTGGTTTTCTACATTTGACACAT |          |          |
| TAS2R30| F: GGTGTTATTACTACATTGGTATG | 603      | 603      |
|        | R: AAAGACGTTCTTCCCTACGAC |          |          |
| TAS2R31| F: CATGGCTAATTTCATGGGACGC | 661      | 661      |
|        | R: GATATCATTATGGCAAGAATGAAAC |          |          |
| TAS2R38| F: ACAGTGATGGGCTCTGCTG | 766      | 766      |
|        | R: GTCCTCTCCTTTCATGGCAC |          |          |
| TAS2R39| F: TGTGCTGATTTCTTCCACTCCCTTA | 841      | 841      |
|        | R: ATTTAGTTGTGCTGGCAGGGT |          |          |
| TAS2R40| F: AGAGTGCACTCTGCAGCTTCT | 685      | 685      |
|        | R: GAGGATGGAAGAGTACGCTGGTGCC |          |          |
| TAS2R41| F: GGTGCTGCTCCTTGGGATATAG | 738      | 738      |
|        | R: TGAGATGAGGATGAGGATG |          |          |
| TAS2R42| F: ATGCCACCCGGATGACCA | 871      | 871      |
|        | R: GCTGCTGCTTCCGCAAGTAGE |          |          |
| TAS2R43| F: GTGCCTGGAGGCTGTTTC | 698      | 698      |
|        | R: TCTTGGTTTCTCCTAACGAGC |          |          |
| TAS2R45| F: CTCCTCTTGGCAAAATGTGTC | 709      | 709      |
|        | R: GAACCGCGGTTGGCTGAAGAAA |          |          |
| TAS2R46| F: GAGTTGAACTCGCTCTTCCTTAAAC | 606      | 606      |
|        | R: ATAGGAGTGAATGCAATGCTTC |          |          |
| TAS2R50| F: GGTAATTTTCTATGGCTGGTGGAAGG | 710      | 710      |
|        | R: CCTGCTAAATCTGAGACACAGCTTGGG |          |          |
| TAS2R60| F: CAGGCATAGGCTCTTCTGCTG | 748      | 748      |
|        | R: CCCCACACCAGAATTTAAAGTCC |          |          |
| ABL1   | F: AGGATCTGACTTTGGAGGCC | 793      | 193      |
|        | R: CCCCAGGTTGATTAGTACCTGAAGAC |          |          |
| KRT10  | F: CCCCATGACTTTGCGCCTGG | —        | 290      |
|        | R: CAGGGATTTCTCAGGGC |          |          |

(Continued)
exposure has acute and long-term effects and our characterization of sun exposure based on location refers only to presumed long-term exposure. Future studies should focus on determining how any of these factors including sex might directly alter \( \text{TAS2R} \) expression in the skin.

The role of bitter receptors in the skin may become apparent after exploring the most highly expressed receptors and their known agonists. Some TAS2R proteins are promiscuous and bind to a wide variety of substances, whereas others have more specificity and bind to one or a few known substances. The protein products of \( \text{TAS2R5} \) and \( \text{TAS2R20} \), two of the most highly expressed genes in the GTEx data set, are narrowly tuned and recognize one to three of 104 known bitter compounds [10]. TAS2R4, the product of \( \text{TAS2R4} \), another highly expressed gene in this study, is intermediate and binds to 6–16 known bitter compounds. Finally, the

| Primer | Sequences (5'→3') | DNA (bp) | RNA (bp) |
|--------|-------------------|----------|----------|
| GNAT3  | F: TCTGGGTATGGCCAATGA | — | 386 |
|        | R: GGCCAGTGATATTCTGAAA | | |

The oligonucleotide sequences and the corresponding amplicon sizes are given for genomic DNA and cDNA. F, Forward; R, reverse; bp, base pairs.

Fig 4. Quantification of bitter taste-related gene expression—qPCR results from cDNA of skin samples after amplification for genes of interest. cDNA was amplified with primers for GNAT3, KRT10, and the 25 TAS2R genes. Data were standardized to the housekeeping gene \( \text{GAPDH} \), and \( 2^{\Delta\Delta Ct} \) was calculated. Results were plotted with individual values in gray and mean across all subjects in red (n = 9). Data points for the FP sample are in blue.

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Determining the cellular expression of TAS2R proteins in skin is an important next step. Bitter receptors are typically expressed in cells known to have chemosensory functions and these cell types are typically sparsely distributed (nose, gut, and tongue). Although we do not know which cell type in human skin expresses TAS2R mRNA, previous studies suggest that they may be in the epidermis, and potentially expressed by keratinocytes [10, 11]. There may also be previously uncharacterized cell types in human skin similar to solitary chemosensory cells that express bitter receptors [19], where we speculate that they may function in innate immunity, wound healing, and/or differentiation. Future studies should attempt to determine the localization of TAS2Rs in skin potentially through immunocytochemistry, which would require validating human TAS2R antibodies, or in situ mRNA hybridization.

**Materials and methods**

**Sample collection and DNA/RNA extraction**

Staff at the University of Pennsylvania Department of Dermatology collected healthy skin from 15 Mohs surgery patients for this study (n = 4 female/11 male; mean age, 62 ± 11.24 years). The Mohs procedure is used to remove cancerous skin and requires removal of additional healthy skin to facilitate proper closure of the wound [13]. We received this additional healthy skin on the day of its removal. The information obtained about each subject was provided by the department and is summarized in Tables 1 and 2. Removal location was provided and based on that information as well as the proximity to cancerous skin we presumed that all samples should be considered sun-exposed. We also obtained one FP biopsy from the tongue of a separate donor as a positive control for TAS2R expression. FP were removed from the surface of the tongue using curved spring micro-scissors [20]. The papillae and skin tissue (0.5 mg) were mechanically homogenized and DNA and RNA was extracted using the Zymo Duet DNA/RNA MiniPrep Plus kit, following the protocol for solid tissue. DNA and RNA was quantified with the Thermo Fisher Scientific NanoDrop 1000 Spectrophotometer and measured RNA degradation through RNA integrity number equivalents generated by the Agilent TapeStation and High Sensitivity ScreenTape Assay. The RNA underwent an extra DNAse treatment using the Thermo Fisher TURBO DNA-free Kit; RNA (100ng) in water (5 μL) was
then reverse transcribed into cDNA using the NuGEN Ovation RNA Amplification System V2 protocol, purified with the QIAquick PCR Purification Kit, and again quantified. The Institutional Review Board at the University of Pennsylvania approved the collection of skin biopsies for this use.

**Primers and PCR amplification**

Primer sets for *KRT10* and *GNAT3* were designed using the NCBI Primer-BLAST tool. The *ABL1* primers are designed to span introns, leading to expected bands at 793 base pairs for genomic DNA and 193 base pairs for cDNA[12]. Primer sets for all 25 *TAS2R* genes have been previously published [11]. PCR reactions using primers listed in Table 3 (Invitrogen, Carlsbad, CA, USA) were performed according to the Invitrogen Platinum Taq Green Hot Start DNA Polymerase protocol with a 1 µL template. The total amount of genomic DNA from each sample was 10 ng, and the total amount of cDNA from each sample was 50 ng. A StepOne Thermocycler was used according to the following profile: one cycle of 4 min at 94°C; 40 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C; one cycle of a final hold at 4°C. Fragments were

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*Fig 5. Expression levels of TAS2R genes from RNA-seq obtained from the GTEx database. Data are plotted with individual RPKM values in gray points and mean across all samples in red lines (N = 914).*

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detected by staining with SYBR Green Safe. The PCR products were electrophoresed on a 1.0% gel in TAE buffer.

**Real-time qPCR**

Real Time qPCR reactions were performed in 10 μL of water in a 384-well plate according to the TaqMan Fast Advanced Master Mix protocol with 1 μL template and run in triplicate. The total amount of cDNA from each sample was 50 ng. Primers for skin-specific markers, TAS2Rs, and a pre-developed endogenous control, GAPDH were used. PCR reactions were performed with the QuantStudio 12K Flex Real-Time PCR machine and amplification was evaluated by comparative analysis based on cycle threshold [21]. Graphs were generated using GraphPad Prism 7 (La Jolla, CA, USA).

**GTEx database analysis**

RNA-seq data from 914 post-mortem tissue samples were provided by the GTEx project (Tables 2 and 4), with information about each sample, including the age and sex of the tissue donor, and tissue type (sun-exposed skin from lower leg or sun-unexposed skin from suprapubic region). Details on skin sample removal, sectioning, and preservation can be found in the GTEx Tissue Harvesting Work Instruction (https://biospecimens.cancer.gov). For the 25 bitter receptor genes from 914 samples, the gene expression RPKM values were normalized for all samples of the same tissue type. Due to the heterogeneity of variance between the genes, we used the non-parametric Kruskal-Wallis test to detect differences in the distribution of expression levels based on effects of sun exposure and of sex within each tissue type (S1–S3 Tables). For analysis of effects of sun exposure, only data from the 299 subjects that donated both types.
of samples were included. For effects of sex in skin from the lower leg, all 508 tissue samples were included, and from the suprapubic area, all 406 tissue samples were included. Data for sun exposure effects and sex were analyzed in R version 3.4.2, and graphs were generated in GraphPad Prism 7. Effects of age were analyzed via correlation and plotted in R (version 3.4.2) and R-studio (version 1.0.136). We deposited a data analysis script based in R on Github (https://github.com/DanielleReed/TAS2R38).
Supporting information

S1 Fig. Gene expression of ABL1. PCR was performed with genomic DNA from skin (gDNA), a mixture of genomic DNA and cDNA from skin (Mix), cDNA from fungiform papillae (FP), and cDNA from 14 skin samples (001–015). Water was used as a no-template control. The larger band at 793 base pairs (bp) includes introns, and the smaller band at 293 bp does not contain introns. Genomic DNA was used as a positive control for the larger band size. A mix was used as a positive control for both bands. The smear at FP is likely caused by nonspecific binding.

S2 Fig. Gene expression of TAS2R1. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 813 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S3 Fig. Gene expression of TAS2R3. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 575 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S4 Fig. Gene expression of TAS2R4. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 749 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S5 Fig. Gene expression of TAS2R5. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 667 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S6 Fig. Gene expression of TAS2R7. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 658 bp. The experiment was replicated.
(bottom panel) because taste receptors are not abundant and can have variable results.

S7 Fig. Gene expression of *TAS2R8*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 723 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S8 Fig. Gene expression of *TAS2R9*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 807 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S9 Fig. Gene expression of *TAS2R10*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 783 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S10 Fig. Gene expression of *TAS2R13*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 742 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S11 Fig. Gene expression of *TAS2R14*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 796 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S12 Fig. Gene expression of *TAS2R16*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 419 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S13 Fig. Gene expression of *TAS2R19*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 606 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S14 Fig. Gene expression of *TAS2R20*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 770 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S15 Fig. Gene expression of *TAS2R30*. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was
used as a no-template control. The expected band size is 603 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S16 Fig. Gene expression of TAS2R31. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 661 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S17 Fig. Gene expression of TAS2R38. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 766 bp. Multiple bands are likely because of non-specific binding. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S18 Fig. Gene expression of TAS2R39. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 841 bp. The experiment was replicated, but results were omitted because of non-specific binding.

S19 Fig. Gene expression of TAS2R40. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 685 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S20 Fig. Gene expression of TAS2R41. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 738 bp. Multiple bands are likely because of non-specific binding. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S21 Fig. Gene expression of TAS2R42. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 871 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S22 Fig. Gene expression of TAS2R43. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 698 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results.

S23 Fig. Gene expression of TAS2R45. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 709 bp. Multiple bands are likely because of non-specific binding. The experiment was replicated (bottom panel) because taste
receptors are not abundant and can have variable results.

(S24 Fig. Gene expression of TAS2R46. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 606 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results. (JPG))

(S25 Fig. Gene expression of TAS2R50. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 710 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results. (JPG))

(S26 Fig. Gene expression of TAS2R60. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The expected band size is 748 bp. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results. (JPG))

(S27 Fig. Gene expression of GNAT3. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The primer set is intron-spanning, so there is no expected band size for genomic DNA, while there is an expected band size of 386 bp for cDNA. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results. (JPG))

(S28 Fig. Gene expression of KRT10. PCR was performed with genomic DNA from skin (gDNA), cDNA from fungiform papillae (FP), and cDNA from nine skin samples. Water was used as a no-template control. The primer set is intron-spanning, so there is no expected band size for genomic DNA and an expected band size of 290 bp for cDNA. The experiment was replicated (bottom panel) because taste receptors are not abundant and can have variable results. (JPG))

(S1 Table. Kruskal-Wallis test statistics for GTEx data comparing effects of presumed sun exposure for each gene of interest (N = 598). (PDF))

(S2 Table. Kruskal-Wallis test statistics for GTEx data comparing effects of sex for each gene of interest in not sun-exposed tissue (N = 406). (PDF))

(S3 Table. Kruskal-Wallis test statistics for GTEx data comparing effects of sex for each gene of interest in sun-exposed tissue (N = 508). (PDF))

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