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The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12

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The perceived taste intensities of quinine HCl, caffeine, sucrose octaacetate (SOA) and propylthiouracil (PROP) solutions were examined in 1457 twins and their siblings. Previous heritability modeling of these bitter stimuli indicated a common genetic factor for quinine, caffeine and SOA (22–28%), as well as separate specific genetic factors for PROP (72%) and quinine (15%). To identify the genes involved, we performed a genome-wide association study with the same sample as the modeling analysis, genotyped for approximately 610 000 single-nucleotide polymorphisms (SNPs). For caffeine and SOA, no SNP association reached a genome-wide statistical criterion. For PROP, the peak association was within *TAS2R38* (*rs713598*, A49P, $P = 1.6 \times 10^{-104}$), which accounted for 45.9% of the trait variance. For quinine, the peak association was centered in a region that contains bitter receptor as well as salivary protein genes and explained 5.8% of the trait variance (*TAS2R19*, *rs10772420*, R299C, $P = 1.8 \times 10^{-15}$). We confirmed this association in a replication sample of twins of similar ancestry ($P = 0.00001$). The specific genetic factor for the perceived intensity of PROP was identified as the gene previously implicated in this trait (*TAS2R38*). For quinine, one or more bitter receptor or salivary proline-rich protein genes on chromosome 12 have alleles which affect its perception but tight linkage among very similar genes precludes the identification of a single causal genetic variant.

INTRODUCTION

In 1934, Professor R.A. Fisher began a series of studies in the Galton Laboratory at Oxford aimed at understanding the inheritance pattern of bitter taste perception for the then-new compound phenylthiocarbamide (PTC). These studies were interrupted by the start of World War II but the record cards were preserved and later analyzed by Dr D.S. Falconer, who published a popular paper about individual differences (1). What has been overlooked, then and now, is that people differ markedly in their ability to taste many other bitter compounds besides PTC and related structures. In Dr Fisher's study, the ability to perceive the bitterness of quinine was also tested and found to be markedly different among subjects and yet these results were overshadowed by his interest in the newer compound. In fact, sensory differences for many bitter compounds, i.e. PROP (propylthiouracil, a close chemical relative of PTC), caffeine, sucrose octaacetate (SOA) and quinine are heritable (2–4). For PROP and PTC, alleles of one of the 25 bitter receptors (*TAS2R38*) explain most of the genetic variation (5–7) although other modifier loci may exist (8–10). We have shown that, for caffeine, SOA and quinine, a common genetic factor accounts for 22–28% of the phenotypic variance, and a quinine-specific factor accounts

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for 15% of variation (4). However, unlike PROP and PTC, the genes responsible for variation in the perceived bitterness of these compounds have not been identified. To find these genes, we conducted genome-wide analyses in twins and their siblings (discovery sample) (11) and tested the associations in a second sample of twins (replication sample). We included PROP as a bitter taste stimulus to serve as a positive control because its genetic architecture is well understood.

RESULTS

Table 1 lists the sample sizes, age, sex and other details for the discovery and replication subject populations.

For caffeine and SOA, no single-nucleotide polymorphisms (SNPs) reached our genome-wide significance threshold of 1.136×10^{-8} (see reference 12 for rationale for threshold; Fig. 1). Previous research has demonstrated that a person's thresholds or perceived intensity ratings for caffeine, SOA and quinine are correlated (4,13,14), suggesting that these individual differences arise from a common mechanism. However, we found no overlapping genetic associations for all three taste stimuli. For the PROP solution, 157 SNPs (30 of which were genotyped) reached this criterion *P*-value, with *TAS2R38* on chromosome 7 at the epicenter. The strongest signals in this region were observed at *rs713598* ($P = 1.6 \times 10^{-104}$) and *rs10246939* ($P = 1.1 \times 10^{-101}$). This region accounted for a maximum trait variance of 45.9%. When this association was accounted for (i.e. after conditioning on the genotyped peak SNP, *rs10246939*), there was little evidence for a nearby secondary peak (chromosome 7: rs13238628, $P = 2.1 \times 10^{-6}$). The two highest secondary peaks identified after conditioning on the peak SNP may be due to chance (chromosome 2: *rs4141835*, $P = 6.8 \times 10^{-7}$; chromosome 7: *rs4727180*; $P = 2.1 \times 10^{-6}$). The results for PROP-saturated paper overlapped with those for the solution, and all SNPs were within or near *TAS2R38* (rs1726866; $P = 2.10 \times 10^{-42}$). A region near a previously identified modifier locus (chromosome 5; *TAS2R1*) approached but did not meet the statistical criterion for genome-wide significance (*rs6867567*; $P = 2.20 \times 10^{-7}$). For other details, see Supplementary Material, Table S1.

For quinine, 19 SNPs (12 of which were genotyped) from chromosome 12, near and within a cluster of bitter receptor and two salivary protein genes, were associated with bitterness perception. Eleven of the associated markers were within the introns of two proline-rich protein genes, and the other SNP coded for an arginine-to-cysteine substitution at amino acid 299 (R299C) in the bitter receptor *TAS2R19* (*rs10772420*, R299C, $P = 1.8 \times 10^{-15}$; formerly known as *TAS2R48*) (Fig. 2). This region accounted for a maximum trait variance of 5.77%. After conditioning on the peak SNP (*rs10772420*), no secondary peaks reached the statistical criterion for genome-wide significance. We tested this association for quinine in an independent group of twins from the general population, collected as a part of a larger study on the genetics of taste perception conducted at a festival held in Twinsburg, OH, USA. One SNP (*rs10772420)* was genotyped, chosen from a subset of markers from the discovery sample because it was tightly associated with the ratings of quinine intensity and because it created an amino acid change in a bitter receptor (TaqMan, Applied Biosystems). The results indicated that the A allele of the genetic variant *TAS2R19* R299C (*rs10772420*) was associated with more intense quinine perception [$F(2,66) = 13.8$, $P = 0.00001$] with the same direction of effect and the same allele associated with increased quinine sensitivity in both the discovery and replication samples (Fig. 3).

Table 1. Discovery and replication sample characteristics

Characteristic	Discovery	Replication
n	1457	73
Males/females	671/786	16/57
Age (mean + standard deviation)	18 + 2	42 + 17
Age range (years)	11–25	21–82
Race/ethnicity	Caucasian	Caucasian
Number of twins (MZ/DZ)	405/847	52/21
Number of siblings	205	0
Country ascertained	Australia	USA

MZ, monozygotic; DZ, dizygotic.

DISCUSSION

For quinine, the SNPs identified in the genome-wide association study implicate two orally expressed gene families: proline-rich proteins that are secreted in saliva (15) and bitter receptors that are found in taste receptor cells (16,17). These two types of genes have many family members and many alleles (18,19). Alleles from these gene clusters are in strong linkage disequilibrium (LD), thus the particular gene responsible for this taste trait cannot be readily identified by genetic association alone. Cell-based expression assays are useful for identifying receptor alleles and their functional responses and have been used to unequivocally identify alleles of a bitter receptor gene associated with taste insensitivity for other ligands (6). However, the assays conducted to date with quinine suggest that many bitter receptor genes from the cluster studied here respond to quinine (*TAS2R7, 10, 14, 46* and *43*) (7) although not all receptors could be tested due to technical limitations, e.g. *TAS2R19*. Also the specificity of these quinine responses cannot be adequately assessed at this time due to the ability of quinine to activate cells indirectly (20). Thus far, specific alleles have not been evaluated for their response to quinine. Furthermore, candidate bitter receptors may heterodimerize within native taste cells, adding to the complexity of identifying specific alleles (21). Although the identification of particular genes is difficult, this region is also supported by comparative studies. Quinine sensitivity in mice maps to the homologous region identified here (22–25).

For PROP, the region known to be associated with its perception was also supported in this analysis, i.e. *TAS2R38* (6), accounting for almost half of the trait variance. Alleles near a bitter receptor on chromosome 5 previously associated with PROP perception also tended to be associated in this study (8,16).

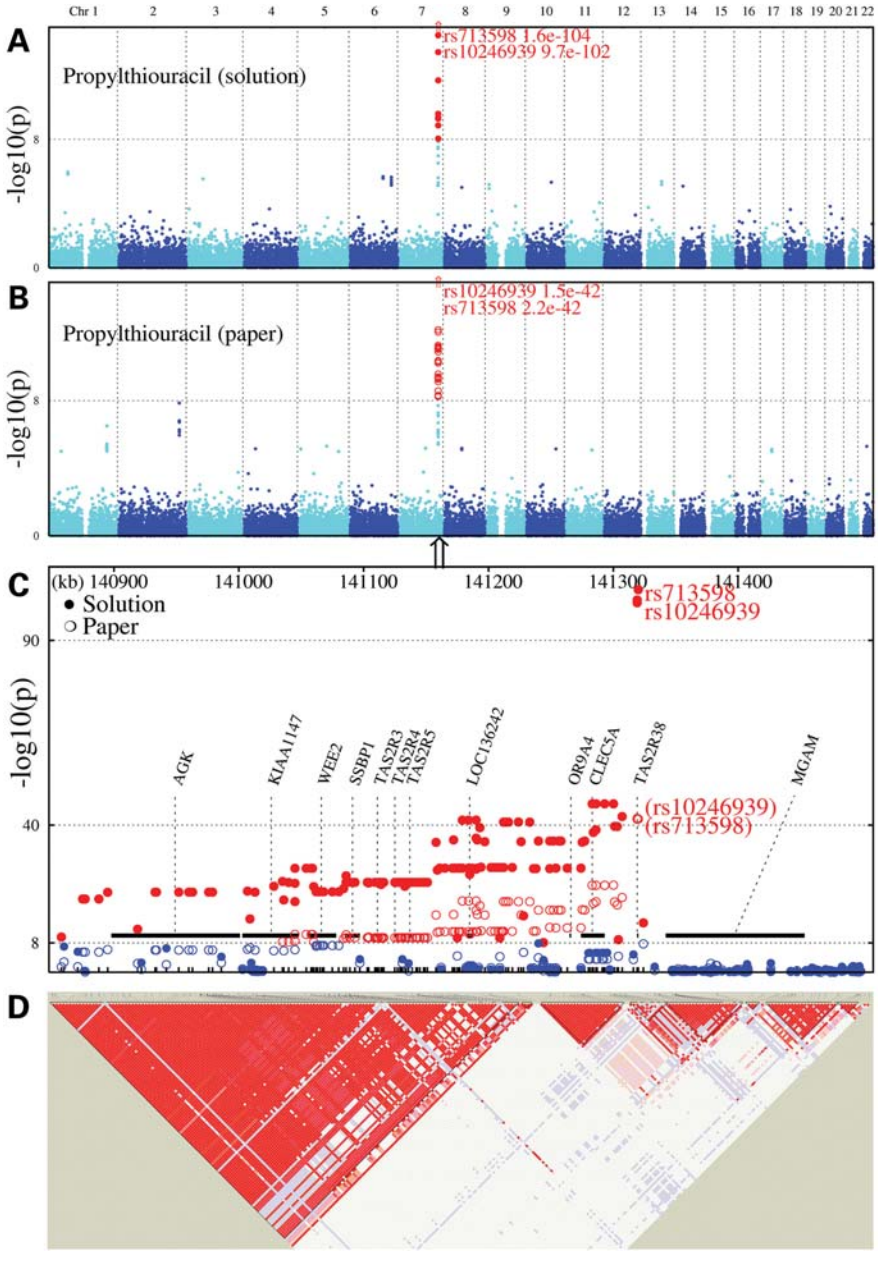

Figure 1. Genome-wide association for PROP perception (**A** and **B**), followed by regional association between 7q34 variants and PROP perception (**C**) and LD among markers for the 7q34 region (**D**). (A) and (B) The observed −log 10 *P*-values by position (Mbp) for bitterness of PROP tasted in solution (A) and tasted within a saturated paper strip (B). The horizontal dotted gray lines show the genome-wide association significance level corrected for the 4.4 independent traits analyzed (calculated using http://gump.qimr.edu.au/general/daleN/matSpD/). The regional association plot between 7q34 variants and PROP perception (C) indicates the location of known genes. Bitterness of PROP tasted in solution is indicated using closed circles and tasted on a saturated paper strip is shown using open circles. (D) Heat-map of the LD in the 7q34 region. The second LD block captures the high LD within the region of peak association. Bitter receptor gene nomenclature has recently changed and gene alias are available online (http://www.ensembl.org/index.html).

Bitter receptors have been strongly selected during human evolution (26–29). Although the reason is not understood, flexibility within a population's ability to tolerate or avoid bitter foods might be useful in some environments and circumstances (e.g. to discover which plants are poisonous or, conversely, to tolerate nutritious plants that contain toxins) (30). However, the focus on oral toxin detection may be too narrow. Recently, the scope of the TAS2Rs and their role in detecting chemicals have been expanded to include the gut (31–34) and airways (35,36). Of particular note are two studies showing that bitter receptors respond to the chemicals secreted by bacteria and thus help the body fight

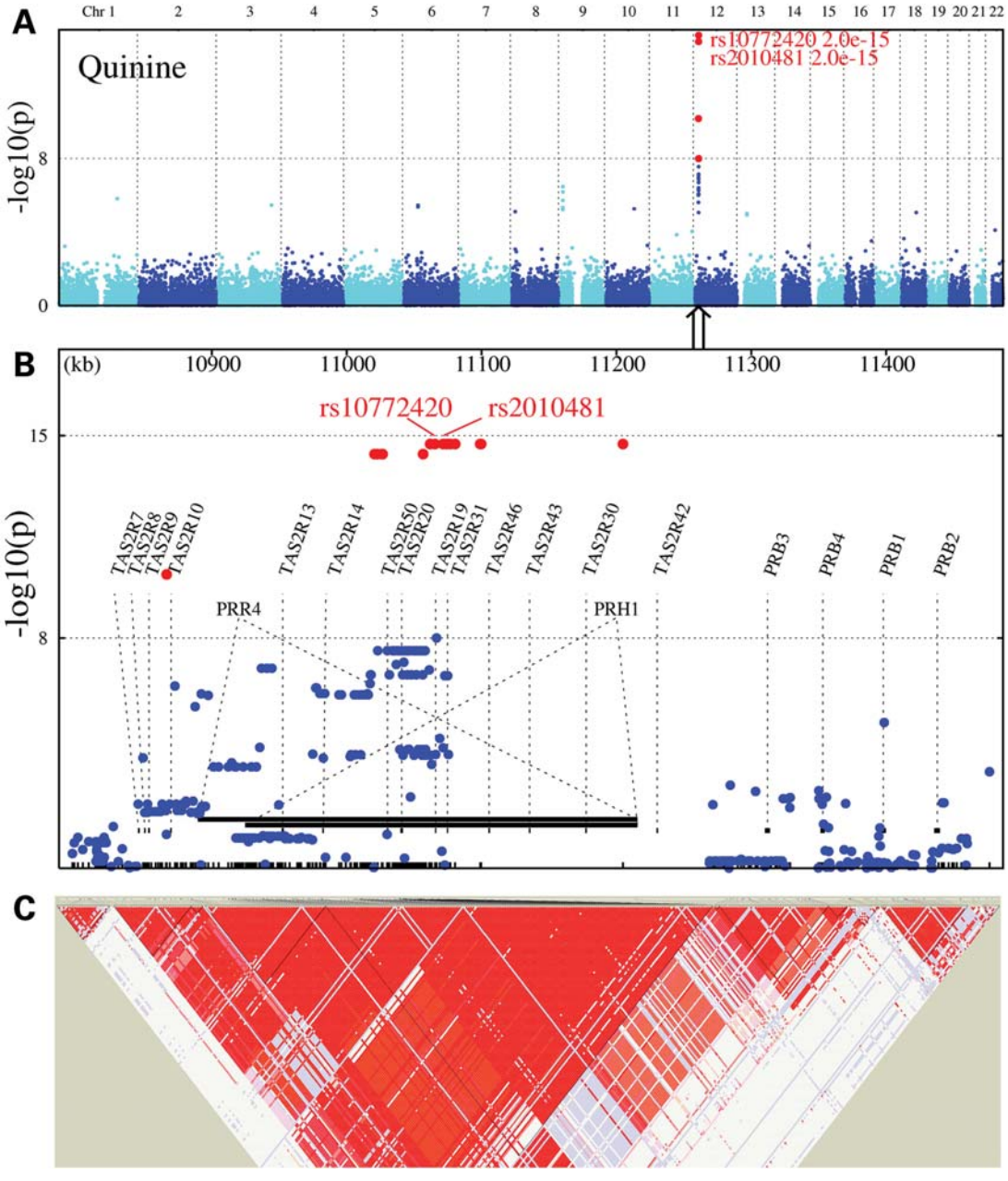

Figure 2. Genome-wide association for quinine perception (**A**), followed by regional association between 12p13.2 variants and quinine perception (**B**) and LD among markers for the 12p13.2 region (**C**). See Figure 1 for details.

infection, and their alleles have also been recently implicated in human diabetes (37). Thus, TAS2R selection could also be driven by chemicals in the nose, lungs, pancreas or gastrointestinal tract.

MATERIALS AND METHODS

Subjects

For the discovery sample, participants were a subset of adolescent and young adult twins and their singleton siblings (11) who have participated in previous studies of the genetics of skin moles and cognition. The sample for which taste sensitivity results were available consisted of females and males and included monozygotic (MZ) and dizygotic (DZ) twin pairs and their siblings. For the replication sample, experimenters recruited and tested participants at an annual convention of twins, Twins Days Festival, in Twinsburg, OH, USA. Testing occurred at the 2009 festival in August (Table 1). The discovery study was performed with the approval of the Queensland Institute of Medical Research (QIMR) Human Research Ethics Committee, and the replication study was

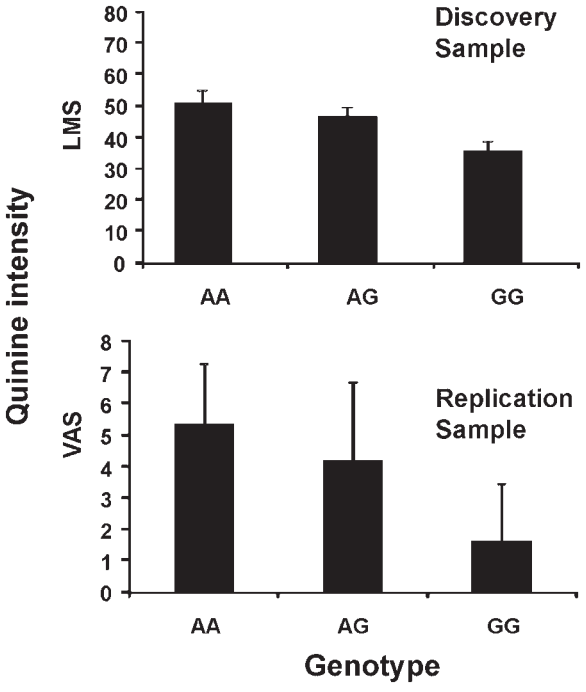

Figure 3. Average bitter taste intensity of quinine as rated by people from the discovery (top) and replication (bottom) samples grouped by their *rs10772420* genotype. LMS, labeled magnitude scale; VAS, visual analogue scale. Values are means and confidence intervals corrected for family relationship (upper panel) or means and standard deviations (lower panel). The *TAS2R19* A allele corresponds to the cysteine amino acid at position 299 and is associated with more intense perception of quinine in both samples.

performed with the approval of the Institutional Review Board at the University of Pennsylvania. Informed consent was obtained from all participants.

Phenotype definition

For the discovery sample, we measured bitter taste intensity for these four compounds by asking participants to rate two 1 ml samples of each solution on a paper-labeled magnitude scale marked with 'no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable', placed semi-logarithmically at 0, 2, 7, 20, 40, 61 and 114 mm, respectively (38). For the discovery sample, the procedures have been described elsewhere (4). They were asked to mark the location on the scale with a pen that best reflected their sensory experience, including regions between labels. The distance from 'no sensation' to their mark was measured in millimeters and used as the dependent variable in statistical analyses. The bitter solutions (0.05 M caffeine, 2.0×10^{-4}M SOA, 1.81×10^{-4}M quinine HCl, 6.0×10^{-4}M PROP) were tested in the following order: SOA, caffeine, quinine, PROP; PROP, quinine, caffeine, SOA. After participants completed the tests with bitter solutions dissolved in water, they used the same scale to rate the taste intensity of a paper strip containing ~1.2 mg of PROP that would dissolve in saliva upon being placed into the mouth. For the replication sample, participants rated their perceived intensity of quinine HCl (7.5×10^{-5}M) on a 7.5 cm visual analogue scale, anchored on the left with 'not at all bitter' and on the right with 'extremely bitter'.

Sample preparation and genotyping

DNA was derived from blood (discovery sample) or from cheek swabs (replication sample). For the discovery sample, genotyping was performed with the Illumina 610-Quad BeadChip, with 529 721 SNPs passing quality control, as outlined previously (39). For the replication sample, the genotyping procedure has been described previously (40).

Statistical analysis

For the discovery sample, to gain the maximum amount of potential information for the association study, genomic coverage was extended to 2.3 M SNPs by imputation using the phased data from the HapMap samples of Caucasian European ancestry (Build 36, Release 22) and MACH 1.0 Markov chain-based haplotyper (41). Quality control filters were applied to the assayed genotypes to restrict the imputation to samples and SNPs with high data quality [i.e. imputation score <0.3 (indicating low imputation confidence; ~3%), a minor allele frequency <0.01 or a Hardy–Weinberg equilibrium score of $P < 10^{-6}$ (~5%)]. Individual SNPs were tested for association with the family-based SCORE test implemented in the software Merlin (42), which accounts for the relatedness of individuals, including MZ twins, after excluding phenotypic outliers, adjusting for age and sex and normalizing each trait (4). The genomic inflation factor (λ) ranged between 0.9982 and 1.0014 (Fig. 4), indicating that potential technical or population stratification artifacts had a negligible impact on the results. For the replication sample, genotype, age, sex, and age by sex interaction were used as fixed factors in a general linear model with the rating of quinine perception as the dependent variable (STATISTICA v 8.0, StatSoft, St Louis, MO, USA). For this sample, only one MZ twin per pair was chosen randomly for inclusion in the analysis.

In addition, from the GWAS, we extracted approximately 9000 SNPs, each 1 Mbp apart, and conducted family-based linkage analysis for (i) the intensity of quinine in solution, (ii) the intensity of PROP in a solution and (iii) the intensity of PROP in saturated filter paper (Supplementary Material, Fig. S1). No linkage was observed for quinine at the region of chromosome 12 [logarithm of odds, (LOD) = 0.73], but this was to be expected given the lack of statistical power (i.e. with 764 quasi-independent sib pairs and QTL additive variance of 23%, we had 20% power at $P = 0.001$). For PROP, we observed a strong linkage signal near the region of the *TAS2R38* gene (LOD = 5.51) which diminishes for intensity ratings of PROP paper (LOD = 1.17). Whereas the QTL additive variance was 51% for the intensity rating of PROP in solution, it accounted for only 24% of the variance in the intensity rating of PROP paper.

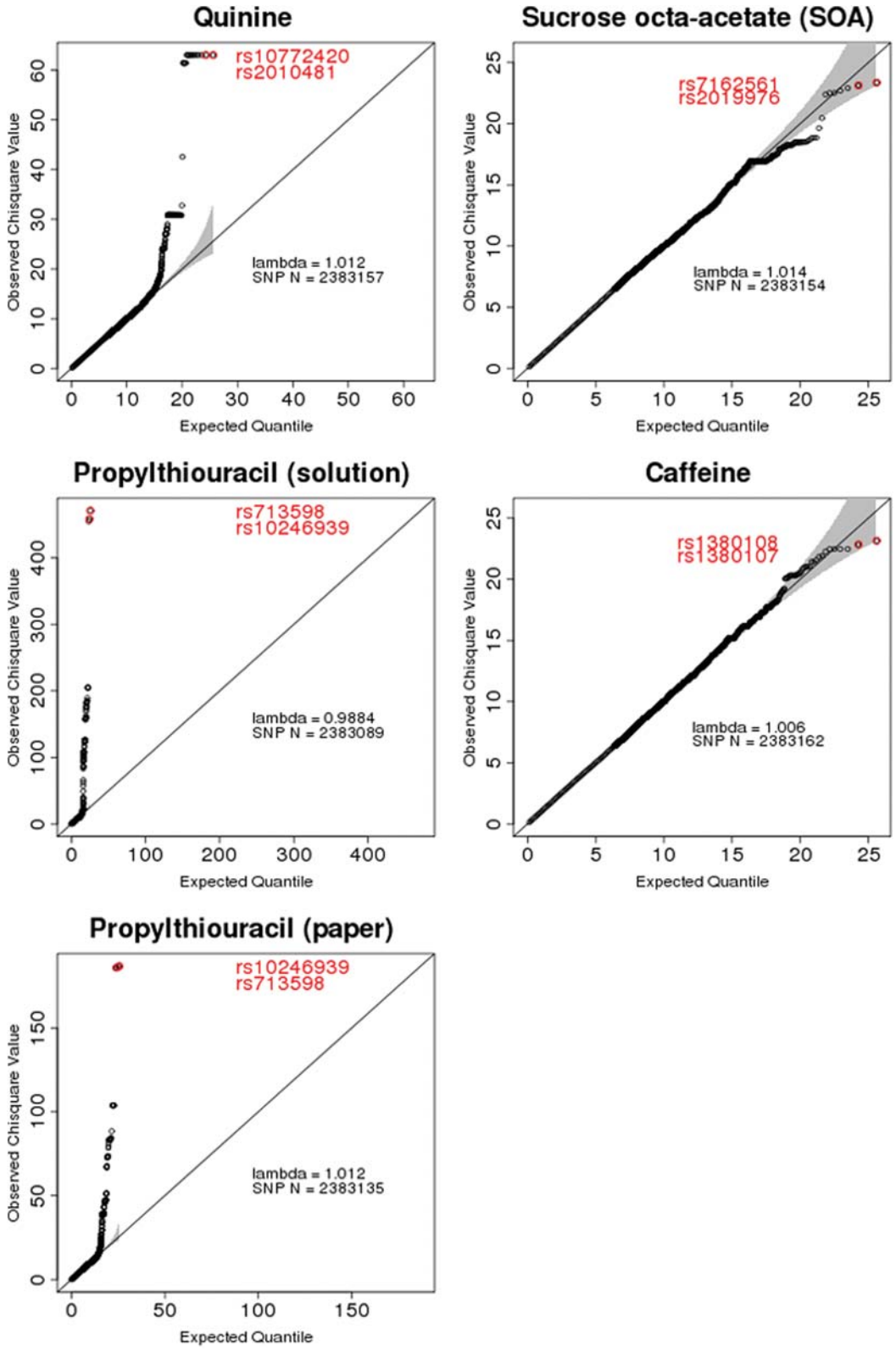

Figure 4. The Q–Q plots for each of the five substances analyzed. The 95% confidence interval is shown in gray. Values lifting above the quinine plot were from a single location on chromosome 12 within and near the *TAS2R19* gene; most of these for PROP (in solution and PROP papers) centered on chromosome 7 within and near the *TAS2R38* gene. The excess of SNPs with small *P*-values is low, and all λ values are near 1.0.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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