The associations between bitter and fat taste sensitivity, and dietary fat intake: Are they impacted by genetic predisposition?

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Abstract

A relationship between bitter and fat taste sensitivity, *CD36* rs1761667 and *TAS2R38* has been demonstrated. However, research is scarce and does not take diet into account. This study aimed to explore associations between genetics, fat and bitter taste sensitivity and dietary fat intake in healthy UK adults. A cross-sectional study was carried out on 88 Caucasian participants (49 females and 39 males aged 35 ± 1 years; body mass index 24.9 ± 0.5 kg/m^2). Bitter taste sensitivity was assessed using phenylthiocarbamide (PTC) impregnated strips and the general Labelled Magnitude Scale. Fat taste sensitivity was assessed by the Ascending Forced Choice Triangle Procedure and dietary intake with a semi-quantitative food frequency questionnaire. Genotyping for rs713598, rs1726866, rs10246939 and rs1761667 was performed. Participants with *TAS2R38* PAV/PAV diplotype perceived PTC strips as more bitter than groups carrying AVI haplotypes (AVI/AVI, $p = 1x10^{-6}$; AVI/AAV, $p = 0.029$). *CD36* rs1761667 was associated with fat taste sensitivity ($p = 0.008$). A negative correlation between bitter taste sensitivity and saturated fat intake was observed ($r_s = -0.256$, $p = 0.016$). When combining the *CD36* genotypes and *TAS2R38* diplotypes into one variable, participants carrying both *TAS2R38* AVI haplotype and *CD36* A allele had a higher intake of saturated fat compared to carriers of *CD36* GG genotype or *TAS2R38* PAV/PAV and PAV/AAV diplotypes (13.8 ± 0.3 vs 12.6 ± 0.5 %TEI, $p = 0.047$) warranting further exploration in a larger cohort.

Keywords: Taste Perception, Diet, rs1761667, CD36, TAS2R38

Abbreviations:

BMI – body mass index; CD36 - cluster of difference 36; FFQ – food frequency questionnaire; FTS – fat taste sensitivity; long-chain fatty acids – LCFA; MUFA - monounsaturated fatty acid; PROP - 6-n-propylthiouracil; PTC – phenylthiocarbamide; PUFA - polyunsaturated fatty acid; SFA – saturated fatty acid; SNP - single nucleotide polymorphism; TEI – total energy intake.

1. Introduction:

Taste sensitivity is an important factor in dietary habit development (Karmous et al., 2018). The five defined human tastes are sweet, sour, bitter, salty and umami (Ikeda, 1909), with a potential sixth taste, fat taste ("oleogustus") recognised recently (Mattes, 2010). The consumption of large amounts of dietary fat constitutes an unhealthy dietary pattern (World Health Organisation (WHO), 2020). Differing taste sensitivity thresholds, which can impact dietary fat consumption, may influence this unhealthy dietary pattern (Duffy & Bartoshuk, 2000; Graham et al., 2021). Research has identified genetic predisposition to all six tastes (Melis et al., 2020), although these have scarcely been studied together.

A wealth of research has reported a clear disparity in the ability to detect bitter compounds such as phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP); a disparity which may be affected by genetics. More specifically, bitter taste sensitivity follows a bimodal distribution, with distinct phenotypes being either non-taster or taster.

To date, various candidate genes have been associated with PROP taste sensitivity, such as the taste receptors from the taste receptor 2 family and the gustin gene, *carbonic anhydrase VI*, (*CA6*) (Melis et al., 2013; Roura et al., 2015). *The bitter taste receptor 2 member 38* (*TAS2R38*) is the most researched receptor to date regarding PROP or PTC taste sensitivity. It contains three coding single nucleotide polymorphisms (SNPs): rs713598 (Pro49Ala), rs1726866 (Ala262Val), and rs10246939 (Val296Ile). These may explain more than 70% of bimodal distribution in PTC taste sensitivity (Kim et al., 2003; Risso et al., 2016). They also create common taster Pro-Ala-Val (PAV) and non-taster Ala-Val-Ile (AVI) haplotypes, observed in over 90% of the Caucasian population (Kim, Wooding, Ricci, Jorde, & Drayna, 2005). In addition, rare haplotypes such as Ala-Ala-Val (AAV), Ala-Ala-Ile (AAI), Pro-Ala-Ile (PAI) and Pro-Val-Ile (PVI) have been identified and may be associated with intermediate sensitivities to PTC and PROP (Risso et al., 2016; Tepper et al., 2008).

Research on genetic determinants of PROP/PTC taste sensitivity has mostly been conducted in Caucasian populations (North Americans or Europeans) that are more likely to be carriers of the non-taster *TAS2R38* AVI haplotype compared to African or Asian populations (Risso et al., 2016). Consequently, Caucasians have also been identified as having lower PROP taste sensitivity than the two above-mentioned populations (Williams et al., 2016; Yang et al., 2020).

Regarding dietary intake, lower bitter taste sensitivity has been associated with higher acceptance and intake of foods with a bitter taste (brassica vegetables, spinach, coffee) (Akella et al., 1997; Drewnowski et al., 1998, 1999), as well as a higher preference for sweet and fatty tasting foods (Duffy & Bartoshuk, 2000), however, these findings are not consistent across studies (O'Brien

et al., 2013; Timpson et al., 2005). Nevertheless, the association between bitter taste sensitivity and intake of foods other than those containing bitter tasting compounds, suggests an interaction with other taste modalities. Considering a larger proportion of bitter non-taster genotypes and phenotypes in Caucasians and the fact these may be associated with diets high in sugar and fat, further research is warranted in this population.

In addition to the above, genetic variants in fat taste sensitivity (FTS) have been reported. There have been two candidate genes of focus within human research; the *cluster of difference 36 (CD36)* and *G-protein coupled receptor 120 (GPR120)* (Costanzo et al., 2019; Daoudi et al., 2015). There is significant evidence of a link between variants within *CD36* and FTS, specifically the rs1761667 (A/G) SNP. This has been associated with FTS (Daoudi et al., 2015; Pepino et al., 2012; Sayed et al., 2015) and dietary fat intake (Fujii et al., 2019; Pepino et al., 2012; Pioltine et al., 2016; Ramos-Lopez et al., 2016). The *CD36* receptor, a membrane protein belonging to the class B scavenger receptor family located in taste bud cells, has been shown to bind to varying concentrations of saturated and unsaturated long-chain fatty acids (LCFA) (Besnard et al., 2016). To date, it is the only defined fat receptor with a high affinity to LCFA (Khan et al., 2020). Individuals with the A-allele have demonstrated reduced protein levels (Ghosh et al., 2011; Love-Gregory & Abumrad, 2011), and therefore have a higher fat detection threshold (hyposensitive) and consequently cannot taste fat as successfully (Melis et al., 2015; A Sayed et al., 2015). These individuals are likely to consume higher quantities of foods containing fatty acids, potentially leading to weight gain (Besnard et al., 2016), although there is paucity in research and what is available is largely heterogeneous (Tucker et al., 2017).

A relationship between bitter and fat taste may be apparent. Prior to the discovery of fat taste and associated receptors, Tepper and Nurse, (1997) described PROP tasters to have a greater ability for oral texture perception through a greater density of trigeminal fibres, thus, a better ability to detect fat. Since this, a relationship between PROP tasters and preference for fat has been demonstrated (Hayes & Duffy, 2007; Tepper & Nurse, 2006). More recently, and in light of this, the *CD36* rs1761667 SNP has been investigated together with PROP taster status and *TAS2R38* haplotypes (Sollai et al., 2019). Although results are consistent regarding the association between fat and bitter taste by both *CD36* rs1761667 and bitter taste *TAS2R38* haplotypes, research is scarce and is yet to be undertaken in a healthy UK cohort comprehensively assessing whether genetic disparities impact dietary intake, alongside taste sensitivity. Therefore, the aim of the current study was to explore the associations between genetics, fat and bitter taste sensitivity and dietary fat intake in healthy UK adults.

2. Methods

2.1 Study design and participants

The participants were healthy Caucasian adults aged 18-65 years and living in the UK. Participants were recruited via word of mouth and internet postings. Exclusion criteria were pregnancy, breastfeeding, chronic medical conditions, food allergies, smoking, lactose intolerance and intake of any medication that may affect taste perception.

At baseline visit, anthropometric measurements including weight (kg), height (m) and waist circumference (cm) were recorded by the research team. Participants provided a 2 mL saliva sample for genotyping and took part in bitter and FTS tests. Participants were asked to refrain from consumption of any food or drink for one hour prior to testing. All participants provided demographic information and completed a food frequency questionnaire (FFQ) administered online (Google Forms).

All procedures involving human participants were approved by the St Mary's and Oxford Brookes University Ethics Committees. Written informed consent was obtained from each participant before the baseline data collection, stating they can withdraw from the study at any point. This study is registered as Genetics of Bitter and Fat Taste at ClinicalTrials.gov NCT04038281.

2.2 Demographic information

Self-reported demographic data (age, sex, ethnicity, income, occupation, and education level) were collected using an online questionnaire (Google Forms).

2.3 Anthropometric measurements

Height (m) [Free Standing Height Measure, SECA GmbH & Co., Hamburg, Germany] and weight (kg) [Portable Scale MS-4203, Marsden Weighing Group, Oxfordshire, UK] were recorded by the research team to the second decimal place. Body mass index (BMI) was calculated using the equation: weight (kg)/ height (m^2) (World Health Organization, 2018).

2.4 Bitter taste sensitivity

The participants rated the intensity of PTC impregnated strip (EISCO labs, Product FSC1031) using the general Labeled Magnitude Scale (gLMS). The gLMS weighted scale labels were: “no sensation” (0), “barely detectable” (1.4), “weak” (6), “moderate” (17), “strong” (35), “very strong” (53), and “the strongest imaginable sensation of any kind” (100) (Roura et al., 2015). Before rating the intensity of the PTC strip, participants were instructed to remember the strongest sensation of any kind they had experienced or the strongest sensation they could imagine happening to them. They were explained these would be deemed as the strongest sensations of any kind on the gLMS scale (Hayes et al., 2013). This was used to guide participants when rating the PTC intensity.

2.5 Fat taste sensitivity

The Oral Fatty Acid Threshold Assessment and Ascending Forced Choice Triangle Procedure was carried out to determine each participant’s oleic acid (C18:1) detection threshold (FTS). The method used, and standard operating procedure followed, is described in full in Haryono, Sprajcer and Keast, (2014). Briefly, each participant was presented with three cups (30 mL UTH-milk based vehicles) in a random order, two controls (oleic-) and one containing oleic acid (oleic+; 0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8, 12, 20 mM). A participant was required to select the oleic+ solution correctly three times at the same concentration to define their threshold. If they were incorrect at any point, a further three cups were presented, one containing the higher oleic+ concentration and two oleic- solutions. Participants were categorised by their FTS result: hypersensitive tasters have a FTS below 3.8 mM, hyposensitive tasters have a FTS above or equal to 3.8 mM and participants who fail to identify the oleic+ sample at the maximum concentration (20 mM) are defined as non-tasters (excluded from analysis) (Haryono et al., 2014; Stewart, Newman, & Keast, 2011).

Testing was conducted on one occasion for each participant. Samples were served at room temperature and presented to participants in individual sections within either the St Mary’s University Nutrition laboratory or Oxford Brookes University sensory laboratory. Red lighting was used to mask visual differences between the samples, nose clips were worn to inhibit olfactory input, textural differences were avoided with the addition of textural agents (gum Arabic and liquid paraffin), and post-ingestive regulation was followed by the sip-and-spit procedure.

2.6 Dietary intake

Habitual dietary intake was assessed with a validated semi-quantitative FFQ (EPIC Norflok). The questionnaires were analysed using the open source, cross-platform tool FFQ EPIC tool for analysis (FETA) (Mulligan et al., 2014) and information on energy and dietary macronutrient intake obtained. More specifically, total carbohydrate, total fat, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA) and total protein were quantified. Intakes of macronutrients were converted into percentage of total energy intake (%TEI) for analyses.

2.7 Single nucleotide polymorphism genotyping

From each participant, a 2 mL saliva sample was collected (SalivaGene Collection Module II; Stratec Molecular GmbH). A stabiliser provided by the manufacturer was added to the saliva sample which was then kept at −20°C until DNA was isolated. Genomic DNA was isolated using a PSP® Saliva-Gene 17 DNA Kit 1011 (Stratec Molecular GmbH) in agreement with the manufacturer procedures. Quality and quantity of the DNA were measured using spectroscopy (Nanodrop, Thermo Fisher, Waltham, MA, USA). Genotyping was then performed using predesigned TaqMan® SNP genotyping assays for the SNPs: rs1761667, rs713598, rs1726866 and rs10246939 and the StepOnePlus thermocycler (Applied Biosystems, CA, USA) with two technical replicates for each sample. The PCR amplification was then completed under the conditions stated by the manufacturer. *TAS2R38* haplotypes, defined by rs713598, rs1726866 and rs10246939, were determined using Haploview software (Barrett et al., 2005).

2.8 Statistical analyses

Hardy Weinberg equilibrium was assessed for all SNPs using Chi-square goodness of fit test. Continuous variables are presented as mean ± standard error of the mean (SEM) or median (interquartile range) and were tested for normality with Shapiro-Wilk test. Categorical variables are presented as absolute (relative) frequencies. Differences in anthropometry, genotype frequencies, bitter and fat taste sensitivity and dietary intake between males and females were tested with an independent samples t-test (with Levene's test for equality of variance), Mann Whitney U or Fisher's Exact test, where appropriate. Individuals who failed to identify the oleic+ solution at 20 mM were defined as non-tasters, therefore have no measurable threshold and were excluded from further

analyses on FTS and measurements of dietary intake by *CD36* genotypes, in line with others (Burgess et al., 2018).

Spearman's correlation was used to explore the associations between bitter and fat taste sensitivity as continuous variables. Kruskal-Wallis H tests were used to test the difference in bitter taste sensitivity between *TAS2R38* diplotype groups and *CD36* genotypes. Bonferroni adjustment were considered for pairwise comparisons. Mann-Whitney U test was used to analyse the differences in bitter taste sensitivity according to the *TAS2R38* rs713598, rs1726866 and rs10246939. Genotypes were dichotomised into carriers of non-taster (Ala, Val, Ile) and homozygous taster alleles (Pro, Ala, Val). Chi-square or Fisher's Exact test, where appropriate, were used to assess the associations between *CD36* genotypes (AA, AG and GG, and AA/AG and GG), *TAS2R38* diplotypes and FTS categories, and to assess the associations between *CD36* genotypes (AA, AG and GG, and AA/AG and GG) and *TAS2R38* diplotypes. Kruskal-Wallis H test was used to explore the difference in fat taste threshold (mM) between *TAS2R38* diplotypes and *CD36* genotypes with Bonferroni adjustment for multiple comparisons.

Spearman's correlation was used to assess the associations between dietary fat intake (total, MUFA, PUFA and SFA) and bitter taste sensitivity, and FTS. One-way analysis of variance (ANOVA) or Kruskal Wallis H, were appropriate, were used to test for differences in dietary intake between *TAS2R38* diplotype groups, and between rs1761667 genotypes (AA, AG and GG). Independent samples t-test (with Levene's test for equality of variance) or Mann Whitney U test, where appropriate, were used to test for differences in dietary intake between rs713598, rs1726866, rs10246939 (carriers of the non-taster and homozygous taster allele), and rs1761667 genotypes (AA/AG and GG) as well as a variable combined of *CD36* genotypes and *TAS2R38* diplotypes (Non-tasters: participants carrying both *TAS2R38* AVI haplotype and *CD36* A allele vs Tasters: carriers of *CD36* GG genotype or *TAS2R38* PAV/PAV and PAV/AAV diplotypes). Participants with AVI/PAV diplotype were grouped with non-tasters considering that larger proportion of our study population carrying this diplotype was deemed a non-taster using the classification by Roura *et al*. (2015) explained below. Finally, two-way ANOVA was conducted to explore the interaction between fat and bitter taster categories on dietary fat intake (total fat, MUFA, PUFA and SFA). For this purpose, PTC ratings were used to categorise the participants into three distinct taster groups. The cut-off criteria were: hyposensitive taster (non-taster) ≤15.5, normal taster >15.5, and hypersensitive taster ≥51 (Roura et al., 2015). Considering a low number of hypersensitive tasters, these were excluded from the analysis. Bonferroni adjustment was used for multiple comparisons.

SPSS was used throughout (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). All tests were two-tailed, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Participant characteristics

Participant characteristics are shown in Table 1. Participants were healthy Caucasians (49 females (56%) and 39 males (44%)) with mean age 35 ± 1 years and BMI 24.9 ± 0.5 kg/ m^2. There were no differences in any of the presented variables or genotype frequencies according to sex, therefore males and females were combined in all analyses (data not shown). No differences in BMI were found between genotypes/diplotypes or bitter and fat taster categories (data not shown). Genotype/diplotype frequency of fat non-tasters can be found in Supplementary Table 1.

The *TAS2R38* and *CD36* SNPs were in Hardy Weinberg equilibrium ($p = 0.825$, $p = 0.573$, $p = 0.573$ and $p = 0.217$ for the rs713598, rs1726866, rs10246939 and rs1761667 respectively). Haplotype frequencies of *TAS2R38* in the study population were: AVI (53%), PAV (42%) and AAV (5%) and allele frequencies of *CD36* rs1761667 were A (61%) and G (39%).

3.2 Taste sensitivity and genetics

There was no correlation between fat and bitter taste sensitivity ($r_s = 0.038$, $p = 0.758$, data not shown, $n = 69$).

As shown in Figure 1, participants carrying PAV/PAV diplotype had higher median ratings of PTC intensity (median (IQR) 31 (30)) compared to participants with AVI haplotype (AVI/AVI, median (IQR) 2 (6) $p = 1x10^{-6}$; AVI/AAV, median (IQR) 4 (14), $p = 0.029$, $n = 88$)). Similarly, those classified as AVI/PAV had higher PTC ratings (median (IQR) 9 (24)) than those homozygous for AVI haplotype ($p = 0.002$, $n = 88$). Carriers of non-taster alleles for rs713598 (Ala), rs1726866 (Val) and rs10246939 (Ile) had lower ratings of bitterness compared to those homozygous for the taster alleles (Pro, Ala and Val, data not shown).

The CD36 rs1761667 was associated with FTS ($p = 0.008$, $n = 69$) when analysed as three genotype groups (AA, AG and GG). Here, a larger proportion of hyposensitive tasters had the AG genotype (55%), this remained significant after Bonferroni corrections were applied (Figure 1). For

exploratory purposes only, non-tasters were included in further analysis, results were consistent (p = 0.033; Supplementary Figure 1), however this was no longer significant after Bonferroni correction applied. When genotypes were combined by variant allele (AA/AG, and GG), a larger percentage of participants carrying the A allele (67.2%) were classified as hyposensitive tasters compared to those homozygous for the G allele (p = 0.013, n = 69, data not shown). Similar was observed when fat taste threshold was treated as a continuous variable (Supplementary Table 3).

There was no association between *TAS2R38* diplotypes and *CD36* rs1761667 (p = 0.622, 0.963, respectively for AA, AG and GG, and AA/AG and GG, n = 88). There was also no difference in PTC ratings of bitterness according to *CD36* rs1761667 genotypes (p = 0.782, 1.000, respectively for AA, AG and GG, and AA/AG and GG, n = 88) or *TAS2R38* diplotypes and fat taste categories (p = 0.384, n = 69). There were no differences in fat taste threshold between *TAS2R38* diplotypes (Supplementary Table 3).

3.3 Associations between genetics, taste sensitivity and diet

As shown in Figure 2, the ratings of PTC intensity were negatively correlated with SFA (%TEI) (r_s = -0.256, p = 0.016, n = 88). There were no correlations between bitter taste sensitivity, total fat, MUFA and PUFA intakes. When excluding participants carrying AVI/AVI diplotype, there was no correlation between PTC bitter taste intensity and dietary fat intake (r_s = -0.229, p = 0.069; r_s = -0.199; p = 0.115; r_s = -0.184; p = 0.145; r_s = -0.166; p = 0.191 for total fat, MUFA, PUFA and SFA respectively). Similarly, there were no correlations between fat taste threshold and any of the presented variables (Figure 3, n = 69). SFA (%TEI) and total fat (%TEI) (r_s = 0.656, p = $3.9x10^{-12}$) and total fat (%TEI) and energy intake (kcal) (r_s = 0.225, p = 0.035) were positively correlated in the total cohort (data not shown).

There were no differences in energy and macronutrient intakes according to *TAS2R38* diplotypes (Table 2, n = 88) or *CD36* rs1761667 (Table 3, n = 69). Similar findings were observed when rare diplotypes AVI/AAV and PAV/AAV were excluded from the analyses (data not shown, n = 78). When analysing individual *TAS2R38* SNPs, there was a significant difference in SFA between rs1726866 and rs10246939 genotypes. Those carrying the non-taster allele for both SNPs (Val and Ile) had higher intake compared to participants homozygous for the taster allele (Ala and Val), both 13.6 ± 0.3 vs 12.1 ± 0.6 %TEI, p = 0.032, n = 88. When combining the *CD36* genotypes and *TAS2R38* diplotypes into one variable, participants carrying both *TAS2R38* AVI haplotype and *CD36* A allele had a higher SFA intake compared to carriers of *CD36* GG genotype or *TAS2R38* PAV/PAV and

PAV/AAV diplotypes (13.8 ± 0.3 vs 12.6 ± 0.5 %TEI, p = 0.047, Supplementary table 2, n = 88). Similar was observed when only *TAS2R38* combined diplotypes were compared (AVI/AVI, AVI/AAV, AVI/PAV vs PAV/PAV, PAV/AAV, data not shown).

Finally, results of the two-way ANOVA showed no interaction between fat (hypo and hyper) and bitter (non-taster and taster) taster categories on total fat (p = 0.111), MUFA (p = 0.474), PUFA (p = 0.220) and SFA (p = 0.218). There were also no main effects of bitter taste category on total fat (p = 0.311), MUFA (p = 0.457), PUFA (p = 0.688) and SFA (p = 0.224). Similarly, there were no main effects of fat taste category on total fat (p = 0.186), MUFA (p = 0.406), PUFA (p = 0.145) and SFA (p = 0.702, Figure 4).

4 Discussion

The aim of this study was to explore the associations between genetics, taste sensitivity (bitter and fat), and dietary fat intake in healthy UK adults. We have demonstrated a difference in bitter taste sensitivity between *TAS2R38* diplotypes and an association between *CD36* rs1761667 and FTS. We did not find an association between *TAS2R38* and FTS, and *CD36* rs1761667 and bitter taste sensitivity. When analysing dietary intake, although there was no association between either *TAS2R38* diplotypes or *CD36* rs1761667 and dietary intake, we did observe a difference in SFA according to *TAS2R38* rs1726866 and rs10246939 genotypes and a negative correlation between bitter taste sensitivity and SFA. Finally, we did not observe an interaction between bitter and fat taste phenotypes on dietary fat intake. However, when combining the *CD36* genotypes and *TAS2R38* diplotypes into one variable, participants carrying both *TAS2R38* AVI haplotype and *CD36* A allele had a higher intake of saturated fat compared to carriers of *CD36* GG genotype or *TAS2R38* PAV/PAV and PAV/AAV diplotypes.

4.1 The associations between *TAS2R38*, bitter taste and diet

We observed differences in the PTC ratings of bitterness according to *TAS238* diplotype groups. Participants with PAV/PAV diplotype had higher ratings than those carrying AVI haplotype and participants classified as AVI/PAV had higher ratings than those homozygous for AVI haplotype. This is in line with previous research where AVI haplotype was associated with bitter non-taster and PAV with a bitter taster phenotype (Bufe et al., 2005; Kim et al., 2005; Tepper, 2008).

In addition to the associations between genetics and taste perception, we also observed an inverse association between bitter taste sensitivity and SFA. Moreover, SFA was positively associated with total fat intake in our study population. This negative association between bitter taste sensitivity and dietary fat intake is in line with previous research reporting higher preference and intake of dietary fat in bitter non-tasters compared to tasters (Choi & Chan, 2015; Duffy, 2004; Tepper & Nurse, 1998). Considering that total fat intake was positively associated with energy intake, a higher intake of SFA may be an indicator of a more energy dense pattern of dietary intake. Since we did not explore dietary patterns, this warrants further research in a similar study population.

The mechanism behind the association between bitter taste sensitivity and dietary fat intake is not entirely clear. It may be that interaction between bitter and fat taste perception exists and this will be discussed later. Considering that the correlation between bitter taste sensitivity and SFA was no longer significant once participants with AVI/AVI diplotype were excluded, this association appears to be driven by genetic predisposition. In this sense, *TAS2R38* is expressed in the gastrointestinal tract where it may regulate the release of satiety hormones and influence the postprandial response to nutrients (Dotson et al., 2010; Rozengurt, 2006). We observed a higher intake of SFA in carriers of the non-taster alleles (Val and Ile) for the rs1726866 and rs10246939 compared to those homozygous for the taster allele (Ala and Val). Similar was observed when *TAS2R38* diplotyes were combined into carriers of the non-taster AVI haplotype and compared to those carrying PAV/PAV or PAV/AAV diplotype. The fact that we did not observe a similar difference in SFA when *TAS2R38* diplotypes were analysed as separate groups may be due to a smaller sub-group sample size when splitting participants into these; this warrants further investigation in a larger sample size study. Interestingly, Dotson *et al.*, (2010) observed an increased eating disinhibition in carriers of the rs1726866 Val, non-taster, allele in their population of Amish women. The authors did not explore dietary intake, however the associations between saturated, total fat and energy intake in our study population suggest that *TAS2R38* may be associated with both eating behaviour, such as eating disinhibition, and a more energy dense dietary pattern. There are number of proposed mechanisms including impaired release of satiety hormones (glucagon-like peptide 1 (GLP-1), insulin) and increased levels of leptin in carriers of the non-taster alleles that warrant further investigation.

Besides the potential effects of *TAS2R38* intestinal expression on hormone signalling, genetic variations in the *CA6* gene may provide an explanation for the association between bitter taste sensitivity and dietary fat intake. Lower fat intake, as %TEI , was observed in UK individuals carrying

the AA genotype of the *CA6* rs2274333 compared to heterozygous AG individuals (Shen et al., 2017). This genotype has been associated with greater bitter taste sensitivity (i.e. PROP super-taster status), through greater fungiform papillae density in AA genotypes compared to homozygous GG genotypes (Melis et al., 2013). Considering that greater fungiform papillae density has also been associated with improved FTS (Zhou et al., 2020), there may be an interaction between *TAS2R38* and *CA6* on dietary fat intake in our study population. These interactions require further research in a similar study population.

Finally, due to the cross-sectional nature of the present study, it is not possible to determine the direction of the association between bitter taste sensitivity and dietary fat intake. Besides the possibility that lower bitter taste sensitivity leads to a higher fat intake, the opposite may also be correct. Jeon *et al*. (2008) suggested that a low-cholesterol diet, likely low in saturated fat, increases the sensitivity of intestinal bitter taste signalling system making the gut more responsive to the presence of bitter tasting compounds. Further intervention studies are, therefore, warranted to explore the cause-and-effect relationship between bitter taste and dietary fat intake.

4.2 The associations between *CD36* rs1761667, fat taste and diet

Furthermore, we observed that the A allele of *CD36* rs1761667 was associated with FTS, specifically a larger percentage of participants carrying the A allele were classified as hyposensitive tasters. This is in line with previous research (Burgess et al., 2018; Chmurzynska et al., 2020; Keller et al., 2012; Melis et al., 2015; Mrizak et al., 2015; Pepino et al., 2012; Amira Sayed & Khan, 2015) and supports that LCFA evoke calcium signalling in gustatory cells expressing *CD36* (El-Yassimi et al., 2008), and that lower protein levels may be related to the A allele hindering ability to detect fat. To date, although research is supportive towards an association between rs1761667 and FTS findings are largely heterogeneous, specifically, regarding ethnicity, which has been shown to modify responses to taste sensitivity (El-Sohemy et al., 2007). Only Melis *et al.*, (2015); Burgess *et al.*, (2018) and Sollai *et al.*, (2019) investigated a Caucasian cohort, similar to ours. Chmurzynska *et al.*, (2020) states recruitment was carried out in Poland, but otherwise does not specify ethnicity of participants. Our results corroborate Melis *et al.*, (2015), and Sollai *et al.*, (2019) but contrast, Burgess *et al.*, (2018) who reported no association between rs1761667 genotype and FTS or perception of fat in the Caucasian sub-group. Results may differ to ours due to Burgess *et al.*, (2018) having a lower sample size (n = 36) than us (n = 69) and Melis *et al.*, (2015) (n = 64), and thus may

have resulted in a type II error. Overall, it is evident the *CD36* rs1761667 A-allele may hinder ability to detect fat in Caucasian participants, although research is scarce. Here it is important to state that other factors may lead to differing taste sensitivity levels alongside rs1761667 genotype. This includes both mechanistic factors, for example rs1527483, another SNP on the *CD36* gene that has been associated with instantaneous orosensory fat taste sensitivity (Plesnik *et al.*, 2018), and interactions between FTS and other tastes, which will be discussed below. Further, an additional factor to consider are fat non-tasters, despite constituting a comparatively small percentage of the population it is unclear whether this sub-population are associated with the same genetic pattern demonstrated by us and others (Burgess et al., 2018; Chmurzynska et al., 2020; Keller et al., 2012; Melis et al., 2015; Mrizak et al., 2015; Pepino et al., 2012; Amira Sayed & Khan, 2015). Such genotypic conclusions cannot yet be drawn since many excluded non-tasters from their analysis (Bajit et al., 2020; Burgess et al., 2018; Karmous et al., 2018; Melis et al., 2020) due to no measurable threshold when undertaking the forced choice triangle method and a small sub-sample. We have included data for the fat non-tasters in our genetic analysis to aid future research comparisons.

It has been stated that a reduced ability to taste fat may lead to greater consumption (Besnard *et al*., 2016). Despite the association found between *CD36* rs1761667 and FTS, we did not observe a difference in dietary intake (total energy, carbohydrate, protein, fat, MUFA, PUFA or SFA). Our findings may be influenced by the majority of our population carrying at least one A allele (81%). To our knowledge, only Graham *et al.*, (2021) and our study assess rs1761667 genotype and dietary intake on a solely Caucasian healthy cohort. Similarly, Pepino *et al.*, (2012) reported no association between genotype and diet, using a mixture of Caucasian and African American (n = 21) participants. Others have reported that the rs1761667 A allele is associated with a higher dietary fat intake. For example, Ramos-Lopez *et al.*, (2016) reported that in participants with chronic hepatitis C the AA genotype is associated with a higher total fat intake (%TEI) and higher SFA (%TEI) ($p < 0.05$), using a 3-day dietary food record. No differences between MUFA and PUFA were found. Similarly, Fujii *et al.*, (2019), using Japanese (n = 495) participants demonstrated the AA genotype was significantly associated with higher total fat, SFA, MUFA, PUFA, omega-3 and -6 intake ($p<0.05$), using a short FFQ. In contrast to this, and contradicting mechanisms associated to the A allele causing a reduced protein expression (Melis et al., 2017), Pioltine *et al.*, (2016) reported the A allele was associated with a decreased intake of total fat (g/day), PUFA and MUFA (% kcal and g/day), fatty foods (portion and g/day), and vegetable oils (mL/day) in Brazilian children and adolescents with obesity, using two 24-hour dietary recalls. It is evident that research regarding dietary intake and rs1761667 genotype is highly heterogeneous, preventing any clear conclusion from being drawn. This warrants further

research in an ethnically homogenous, healthy cohort of adults or children, similar to our own, with consistent dietary collection methods.

4.3 Potential interactions between fat and bitter taste

In our study population bitter and FTS were not correlated. Also, we found neither an association between *TAS2R38* diplotypes and FTS nor *CD36* rs1761667 and bitter taste sensitivity. Our findings contradict other research, reporting an association between the two tastes (Melis et al., 2015; Sollai et al., 2019). Melis *et al.,* (2015), using 64 Italian participants, displayed that perception of fatty acids was associated with rs1761667 *CD36* and that AVI/AVI participants exhibited a 5-fold higher oleic acid threshold than their PAV/PAV counterparts. Later, Sollai *et al.*, (2019), reported similar results but using electrophysiological recordings from the tongue in response to oleic acid in a sample of 35 Italian adults. Similar results have been reported by Karmous *et al.*, (2018), who also displayed a correlation between fat and bitter taste, however in a non-Caucasian (Tunisian) population and by Melis *et al.*, (2020) in patients with inflammatory bowel disease. The fact we did not observe similar associations may be attributed to our study population being UK based and having different allele and haplotype frequencies compared to populations such as Tunisians explored by Karmous *et al.,* (2018). Furthermore, differences in methods of taste sensitivity measurement between studies may also explain discrepancies in results.

None of the aforementioned studies explored the dietary intake of participants. In this sense, we observed an association between genetic predisposition to bitter taste, bitter taste sensitivity and dietary fat intake, where non-tasters have a higher intake of SFA than tasters. Although we did not observe an interaction between bitter and fat taste categories on dietary fat intake, we observed a higher intake of SFA in participants carrying both non-taster *CD36* allele (A) and *TAS2R38* haplotype (AVI) compared to those carrying either taster *CD36* genotype (GG) or *TAS2338* haplotype (PAV/PAV and PAV/AAV). This may suggest that genetic predisposition to hyposensitivity to both fat and bitter taste leads to an increased dietary fat intake, and supports previously observed interactions between the two tastes (Karmous et al., 2018; Melis et al., 2015, 2020; Sollai et al., 2019). It may also corroborate proposed mechanisms whereby *TAS2R38* may be involved in the textural perception of fat, whereas *CD36* may determine the chemosensory detection of fat (Keller, 2012). Considering that higher SFA intake was also observed in carriers of *TAS2R38* haplotype (AVI) compared to those carrying PAV/PAV or PAV/AAV diplotypes it may be that *TAS2R38* is driving these differences. Due to the small sample in our study, we were not able to

determine exact contribution of *TAS2R38* diplotypes and *CD36* rs1761667 in explaining SFA using regression analysis. These results should therefore be considered hypothesis generating and replicated in a larger cohort.

4.4 Strengths and limitations

Besides the fact we comprehensively investigated the associations between genetics, taste and diet, a strength of this study is an ethnically homogenous population enabling a more valid interpretation of genetic association results. However, our population was not homogenous regarding sex, Barragán *et al.,* (2018) reported that sex differences exist in ability to taste. There were no differences between sexes found in any of the variables tested however future research should endeavour to recruit a sex specific cohort or have a sample large enough for sex-specific analyses. Our sample size, although in line with other published research (Karmous et al., 2018; Melis et al., 2015, 2020; Sollai et al., 2019), was low regarding subgroup analysis (Grimaldi *et al.*, 2017). This limits the conclusions that can be drawn and results should be replicated in a larger sample size study.

Moreover, in future studies repeated testing of FTS should be considered. Although some have demonstrated FTS is reproducible (Newman & Keast, 2013), others have demonstrated improvement, specifically within the hypersensitive tasters, over time (Tucker & Mattes, 2013).

Furthermore, the use of PTC filter strips may result in misclassification of participants into bitter tasters and non-tasters (Lawless, 1980). However, more recently, the use of PROP or PTC paper strip has been shown as a valid method to explore genetic predisposition to PTC taste sensitivity (Khataan et al., 2010) and we have used these ratings as a continuous variable in the majority of our analyses. Furthermore, the gLMS may also be more reliable when repeated on multiple occasions (Hayes et al., 2008) and this should be considered in future research. Nevertheless, participants were instructed on the use of the scale, which has been employed in similar studies exploring genetics and bitter taste sensitivity (Yang et al., 2020).

The present study explored the associations between PTC taste sensitivity as a proxy for bitter taste sensitivity and *TAS2R38* receptor as its determinant. PTC is however, only one of the many bitter tasting compounds and may not be a predictor of general bitter taste sensitivity. There are number of TAS2R bitter taste receptors that are activated by different bitter tasting compounds such as caffeine, quinin and saccharin requiring further investigation to gain a more comprehensive understanding of bitter taste variability and its effects on dietary intake (Roura et al., 2015).

Lastly, self-reported dietary intake data, collected via validated FFQ, may be prone to misreporting (Shim, Oh and Kim, 2014). However, to improve accuracy, we selected a population specific FFQ (UK) and expressed macronutrients as % TEI which may improve accuracy of comparisons made (Macdiarmid and Blundell, 1998). Also, although the FFQ used is a validated method to collect dietary consumption over the previous 12 months and has been calibrated using a 24-hour dietary recall, dietary intake may vary over time and FTS has been shown to alter after only weeks of dietary modification (Costanzo et al., 2019; Newman et al., 2016). Therefore, future studies should consider the use of multiple 24-hour dietary recalls to collect dietary intake information.

5 Conclusion

Overall, we confirmed that *TAS2R38* haplotypes determine bitter taste sensitivity and *CD36* rs1761667 is associated with fat taste sensitivity. Lower sensitivity to bitter taste may also lead to a higher dietary intake of fat. Considering the lack of association between bitter taste sensitivity and SFA when excluding participants carrying *TAS2R38* non-taster AVI/AVI diplotype, this appears to be mainly driven by genetic predisposition. Although we did not observe an interaction between bitter and fat taste categories on dietary fat intake, we observed a higher intake of SFA in participants carrying both non-taster *CD36* allele (A) and *TAS2R38* haplotype (AVI) compared to those carrying either taster *CD36* genotype (GG) or *TAS2338* diplotype (PAV/PAV and PAV/AAV). This may suggest that genetic predisposition to hyposensitivity to both fat and bitter taste leads to an increased dietary fat intake. Nevertheless, it warrants further research in a larger cohort employing repeated measurements of bitter and FTS and a combination of dietary consumption methods such as FFQ and 24-hour recalls.

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Declaration of Interest

Yiannis Mavrommatis is a shareholder for Nell Health, a lifestyle genotyping company. Leta Pilic is serving on advisory board of DNAfuel LTD.

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Author Contributions

Catherine Anna-Marie Graham and Leta Pilic: Conceptualization**,** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualisation **Ella Mcgrigor**: Investigation, Data Curation **Megan Brown**: Investigation, Data Curation **Isabelle Jane Easton:** Investigation, Data Curation **Jonathan Nyuma Kean**: Investigation, Data Curation **Verity Sarel**: Investigation, Data Curation **Yasmin Wehliye**: Investigation, Data Curation **Natalie Davis**: Investigation, Data Curation **Nisrin Hares**: Investigation, Data Curation **Deanna Barac** Investigation, Data Curation **Alexandra King**: Writing – Review and Editing **Yiannis Mavrommatis**: Conceptualization, Methodology, Validation, Writing – Review and Editing, Supervision.

All authors have approved the final article.

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Figure legends

Figure 1. Genetics and taste sensitivity

(A) *TAS2R38* diplotypes and bitter taste sensitivity; *total n = 88, PAV/PAV = 13, AVI/PAV = 41, AVI/AVI = 24, PAV/AAV = 3, AVI/AAV = 7, a: different than PAV/PAV (AVI/AVI, p = 1x10^{-6}; AVI/AAV, p = 0.029), b: different than AVI/AVI (p = 0.002). Line represents the median and whiskers min and max values, Kruskal-Wallis H test with Bonferroni adjusted p values.*

(B) *CD36* rs1761667 and fat taste sensitivity, *total n = 69, AA = 29, AG = 29, GG = 11, * p = 0.008, Fischer's Exact test.*

Cluster of differentiation 36 (CD36), Taste 2 receptor member 38 (TAS2R38).

Figure 2. The correlations between bitter taste sensitivity (PTC intensity rating) and dietary fat intake (n = 88); *Phenylthiocarbamyde (PTC), total energy intake (TEI). Spearman's correlation.*

Figure 3. The correlations between fat taste threshold and dietary fat intake (n = 69). Oleic acid concentrations/fat taste threshold was: 0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8, 12 and 20 mM. *Total energy intake (TEI). Spearman's correlation.*

Figure 4. Difference in dietary fat intake according to bitter taster status in A) fat hypersensitive taster (total n = 27, bitter taster = 9, bitter non-taster = 18) and B) fat hyposensitive taster group (total n = 40, bitter taster = 14, bitter non-taster = 26).

Error bars represent ± SEM.

Tables

Table 1. Participant characteristics (n = 88, n = 69). Data presented as mean ± SEM, median (IQR) or absolute (relative) frequencies

	All participants (n = 88)	Fat-tasters only* (n = 69)
Age (years)	35 ± 1	34.7 ± 1.7
BMI (kg/m^2)	24.9 ± 0.5	25.0 ± 0.6
18.5-24.9 kg/m2 n (%)	49 (56)	41 (59)
≥25.0 kg/m2 n (%)	39 (44)	28 (41)
Sex n (%)		
Female	49 (56)	40 (58)
Male	39 (44)	29 (42)
Bitter taste intensity rating m (IQR)	6 (18.5)	8 (27.5)
Fat taste category n (%)		
Hyposensitive	42 (48)	42 (48)
Hypersensitive	27 (31)	27 (31)
Non-taster	19 (21)	-
Energy (kcal)	1656 ± 79	1709 ± 94
Carbohydrate (%TEI)	43.6 ± 0.8	44.4 ± 0.9
Protein (%TEI)	19.3 ± 0.3	19.2 ± 0.4
Total fat (%TEI)	37.5 ± 0.6	37.2 ± 0.7
MUFA (%TEI)	14.1 ± 0.3	14.1 ± 0.4
PUFA (%TEI)	6.6 ± 0.2	6.7 ± 0.3
SFA (%TEI)	13.3 ± 0.2	13.1 ± 0.3

*Body mass index (BMI), Interquartile range (IQR), Median (m), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Saturated fatty acids (SFA), Total energy intake (TEI). * Participants with a defined fat taste threshold.*

Table 2. Energy and macronutrient intakes according to *TAS2R38* diplotypes (n = 88). Data presented as mean ± SEM (One-way ANOVA)

	PAV/PAV (n = 13)	AVI/PAV (n = 41)	AVI/AVI (n = 24)	PAV/AAV (n = 3)	AVI/AAV (n = 7)	p-value
Energy (kcal)	1794 ± 224	1683 ± 137	1512 ± 89	1458 ± 100	1814 ± 289	0.666
Protein (%TEI)	20.4 ± 0.7	19.2 ± 0.6	19.0 ± 0.7	20.7 ± 2	18.9 ± 1.8	0.735
CHO (%TEI)	45. 1 ± 1.5	43.2 ± 1.3	42.3 ± 1.6	48.3 ± 4.5	44.9 ± 2.9	0.631
Total fat (%TEI)	35.2 ± 1.4	37.9 ± 1.1	38.4 ± 1.3	34.0 ± 4.2	37.4 ± 1.3	0.493
SFA (%TEI)	12.5 ± 0.7	13.5 ± 0.4	14.2 ± 0.6	10.0 ± 0.6	12.9 ± 0.6	0.082
MUFA (%TEI)	13.2 ± 0.6	14.4 ± 0.5	14.4 ± 0.6	14.0 ± 2.5	14.4 ± 0.5	0.613
PUFA (%TEI)	6.2 ± 0.3	6.8 ± 0.4	6.6. ± 0.3	6.7 ± 0.7	6.6. ± 0.5	0.928

Carbohydrate (CHO), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Saturated fatty acids (SFA), Taste 2 receptor member 38 (TAS2R38), Total energy intake (TEI).

Table 3. Energy and macronutrient intakes according to *CD36* rs1761667 (n=69). Data presented as mean ± SEM. Kruskal-Wallis, Independent T-test or Man Whitney U test where appropriate.

	AA (n = 29)	AG (n = 29)	GG (n = 11)	AA/AG (n = 58)	p-value[1]	p-value[2]
Energy (kcal)	1803 ± 152	1631 ± 156	1670 ± 150	1717 ± 109	0.416	0.611
Protein (%TEI)	19.1 ± 0.6	19.3 ± 0.7	19.1 ± 0.9	19.2 ± 2.2	0.952	0.786
CHO (%TEI)	44.7 ± 1.4	43.9 ± 1.3	45.2 ± 2.2	44.3 ± 0.9	0.846	0.703
Total fat (%TEI)	36.9 ± 1.3	37.9 ± 1.0	36.0 ± 1.7	37.4 ± 1.7	0.453	0.458
SFA (%TEI)	12.5 ± 0.5	13.8 ± 0.4	12.9 ± 1.0	13.2 ± 0.3	0.156	0.762
MUFA (%TEI)	14.2 ± 0.5	14.2 ± 0.6	13.7 ± 0.7	14.2 ± 0.3	0.869	0.811
PUFA (%TEI)	7.0 ± 0.5	6.5 ± 0.5	6.0 ± 0.3	6.8 ± 0.3	0.514	0.312

Cluster of differentiation 36 (CD36), Carbohydrate (CHO), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Saturated fatty acids (SFA), Total energy intake (TEI), p-value [1] difference in diet between three genotypes (AA, AG, GG), p-value [2] difference in diet between two genotypes (AA/AG and GG).

Figure 1

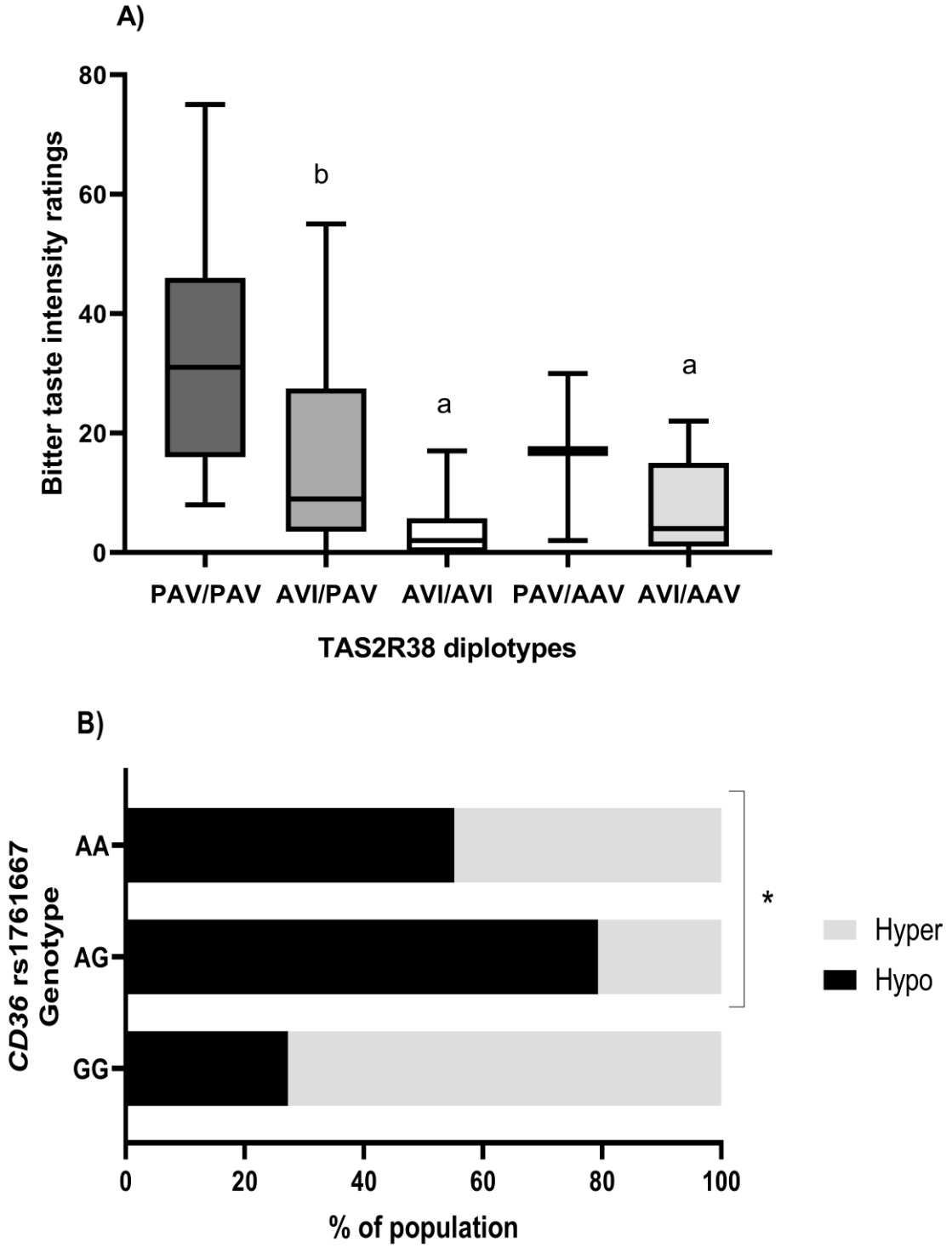

Figure 2

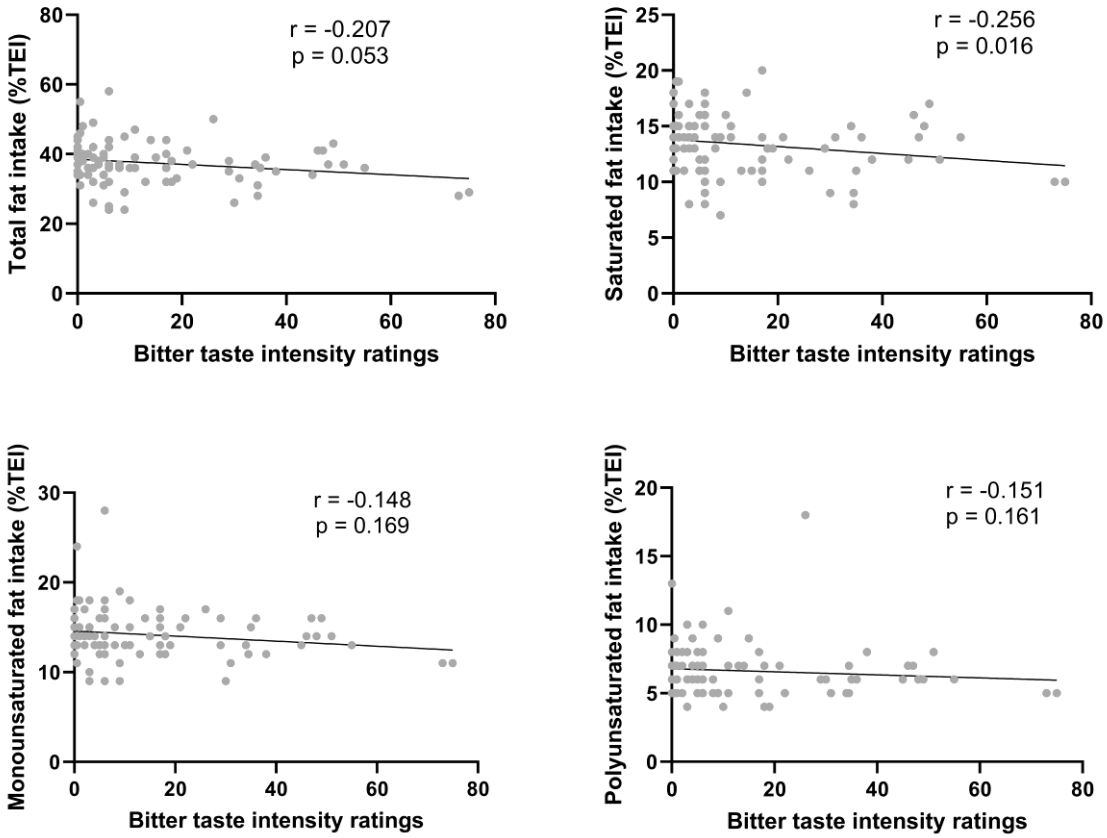

Figure 3

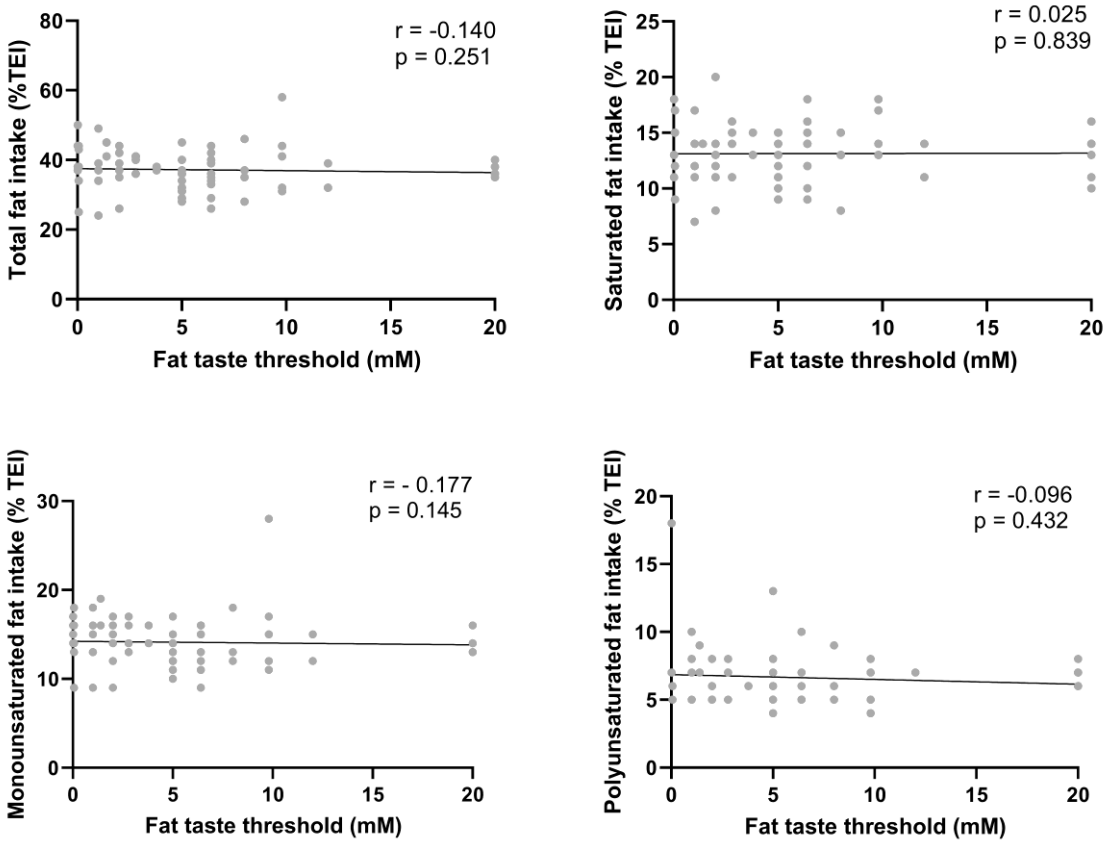

Figure 4