The associations between genetics, salt taste perception and salt intake in young adults

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Abstract

Food liking is one of the main determinants of food intake. Salt taste perception and preference, that play a role in liking of salt, may be genetically determined, although research in humans is scarce. The aim of this study was to explore the associations between genetics, salt taste perception, preference, self-reported salt habit and intake. The participants were young (18-35 years) and healthy adults (32 males and 63 females). Salt taste thresholds were determined with British Standard ISO3972:2011 methodology and salt taste preference by ratings of saltiness and pleasantness of tomato soup with salt concentrations reflecting salt content in foods. Self-reported salt habit was determined by asking participants how salty they usually eat their food and salt intake with two 24-hour 5-step multiple pass recalls. Genotyping for variants in the *SCNN1B* rs239345 and *TRPV1* rs8065080 was performed. Participants homozygous for the minor allele of the rs8065080 had lower ratings of saltiness ($p = 0.008$) and higher ratings of pleasantness of soup ($p = 0.027$) when compared to major allele carriers. Preference for salt in soup was associated with salt habit ($p = 0.003$) and participants with high salt preference had higher salt intake compared to those with low salt preference (2236 ± 261 vs. 1543 ± 107 mg/1000 kcal, $p = 0.017$). *TRPV1* rs8065080 may play a role in salt taste perception and preference, which should be confirmed in a larger sample size study. Hedonic appeal of salty food should be considered when providing personalised advice to change this behaviour.

Key words: Genetics; preference; salt intake; SCNN1B; taste; TRPV1

Abbreviations

AMPM - automated multiple pass method; CVD – cardiovascular disease; DALY – disability adjusted life year; FFQ – food frequency questionnaire; SCNN1B – Epithelial sodium channel 1 subunit beta; SNP – single nucleotide polymorphism; TRPV1 - The transient receptor potential cation channel subfamily V member 1; USDA – United States Department of Agriculture

1. Introduction

Non-communicable disease such as cardiovascular disease (CVD) are among the top ten global causes of death (World Health Organisation, 2018). Unhealthy diets are suggested as key risk factors for such disease accounting for 11 million deaths and 255 million disability adjusted life years (DALYs) worldwide. Specifically, high intake of sodium (hereinafter sodium and salt will be used interchangeably) was among the top three leading dietary risk factors for deaths and DALYs. It was estimated that the mean global sodium consumption in 2017 was 6 g/day, exceeding the recommended intakes of 2.0 g/day by 86% (Afshin et al., 2019).

Food liking, that may be determined by taste perception (taste threshold sensitivity) and preference for a specific taste, is considered as one of the main determinants of food intake and potentially salt (Feeney, O'Brien, Scannell, Markey, & Gibney, 2011). Salt taste sensitivity may be determined by genetic variations in salty taste receptors. One of the first proposed amiloride-sensitive salty taste receptors in the tongue was the epithelial sodium channel (ENaC), involved in transepithelial sodium transport (Bachmanov et al., 2014). Regarding the amiloride-insensitive part of salt taste receptor, one of the candidates is TRPV1 (transient receptor potential cation channel, subfamily V, member 1; formerly named vanilloid receptor subtype 1, or capsaicin receptor). TRPV1 also transduces painful thermal stimuli and is activated by capsaicin (Yang & Zheng, 2017).

Although scarce, research in humans suggests that these receptors may play a role in perception of salty taste. Dias et al. (2013) investigated the associations between genetic variation in the ENaC and the *TRPV1*, expressed lingually, and salt taste threshold and suprathreshold taste sensitivity in young Caucasians. Variants in the beta subunit of the ENaC *SCNN1B* gene together with the *TRPV1* modified suprathreshold salt taste sensitivity. More specifically, individuals homozygous for the A allele of the *SCNN1B* rs239345 had lower suprathreshold salt taste sensitivity than those with either AT or TT genotype. Similar was observed for individuals with the CC genotype of the *TRPV1* rs8065080. Although not clear if the rs239345 is functional, the *TRPV1* rs8065080 is a missense single nucleotide polymorphism (SNP) resulting in amino acid change at position 585, from isoleucine to valine, potentially affecting protein function (Ng & Henikoff, 2006). Studies of its functional effect showed a decreased channel activity in response to two typical TRPV1 stimuli, heat and capsaicin, in TRPV1-Val-585 cells (C allele) compared to TRPV1-Ile-585 (T allele)

(Cantero-Recasens et al., 2010). If this was the case with salt, it may serve as an explanation why participants with CC genotype were reported to have lower taste sensitivity (ie. higher thresholds) (Dias et al., 2013). Despite the associations observed by Dias et al. (2013), the authors highlighted the need for replication of their results. Indeed, the associations between *SCNN1B*, *TRPV1*, salt taste sensitivity and preference have been confirmed recently in cohorts of Spanish and Canadian adults (Barragán et al., 2018; Chamoun et al., 2018). However, little is known about the effects of these genetic variants on the actual salt consumption.

Furthermore, research exploring the relationships between salt taste sensitivity, preference and intake is inconclusive. Matsuzuki, Muto, & Haruyama (2008) found no association between salt taste thresholds and sodium intake in Japanese school children whereas Kim & Lee (2009) showed that the children who reported liking for a Korean high-salt soup/stew had higher thresholds for salt. In adults, Pangborn & Pecore (1982) did not demonstrate a strong relationship between salt intake and taste thresholds while Azinge, Sofola, & Silva (2011) reported a higher urinary sodium excretion in Nigerian adults with higher salt taste thresholds. Piovesana, Sampaio, & Gallani (2013) investigated the relationship between salt taste thresholds and dietary salt intake, evaluated through 24-hour urinary sodium excretion and self-reported measures (discretionary salt, food frequency questionnaire (FFQ), and 24-hour recall) in adult Brazilians. A weak positive correlation was observed between salt taste threshold and salt intake measured with FFQ. Salt intake measured with a urinary biomarker of sodium excretion, a method considered as the gold standard, was not significantly correlated with salt taste thresholds. Finally, Lee et al. (2014) reported how self-reported salt eating habit, but not taste threshold, was a predictor of salt intake in young and healthy Korean adults.

Recently, we showed how blood pressure response to high salt intake in healthy and younger adults may be genetically determined, with salt-sensitive participants exhibiting an average increase in systolic blood pressure of 7.75 mmHg following a high-salt diet. This may be of clinical importance since salt sensitivity of blood pressure is thought to be an independent CVD and mortality risk factor (Pilic & Mavrommatis, 2018). In this sense, determining drivers of salt intake in a healthy population may serve as an avenue to design more targeted approaches to change this dietary behaviour and prevent CVD.

Considering an inconclusive link between salt taste perception and intake, which may be attributed to differences in the study populations or methods employed, these associations should be further explored in a cohort of young and healthy adults where preference may be a driver of salt intake (Pilic & Mavrommatis, 2018). Additionally, the associations between variants in salty taste receptors, *SCNN1B* rs239345 and *TRPV1* rs8065080, explored in context of taste thresholds warrant further investigation in context of the actual salt preference and consumption. Therefore, the aim of the present study was to explore the associations between genetics (*SCNN1B* rs239345 and *TRPV1* rs8065080), salt taste perception (taste threshold sensitivity), preference, self-reported salt habit and intake in young and healthy adults.

2. Methods

2.1. Study design and participants

The participants were predominantly young adult Caucasians (85%) living in the UK, 32 males and 63 females. Participants were recruited through advertisements and Internet postings. Participants were excluded with history of/current chronic disease or the use of any medications to treat chronic disease. In addition, pregnant and lactating women, being underweight (body mass index (BMI) < 18.5 kg/m^2 or obese (BMI > 30 kg/m^2) and participants with an illness that alters taste were also excluded from the study.

During the baseline visit, all participants completed taste threshold determination for salt test and provided a saliva sample for genotyping. Additionally, 74 participants completed a salt taste preference test and provided information on self-reported salt eating habit. On two separate occasions, all participants completed 24-hour dietary recalls which were administered online. All procedures involving human participants were approved by the Institutional Ethics Committee (SMEC_2018-19_007). Written informed consent was obtained from each participant before the baseline data collection, informing they can withdraw from the study at any point. This study is registered as *Factors affecting salt intake in young adults* at ClinicalTrials.gov NTC03871374.

2.2. Baseline measurements

Height and weight were measured at baseline. Demographic data (age, sex, ethnicity, income, occupation and education level) was collected and assessed together with smoking habits and health status information.

2.3. Taste thresholds for salt

Identification of taste thresholds for salt was determined using the British Standard BS ISO3972:2011 methodology. Participants were instructed to refrain from eating or drinking (except water) at least an hour before the testing. Salt taste detection and recognition thresholds were determined using nine graded sodium chloride solutions (4 mmol/l – 49 mmol/l, geometrical ratio of 0.7) with a more detailed protocol described elsewhere (Pilic & Mavrommatis, 2018). The salt taste detection threshold was identified as the lowest concentration of the sample where the participant can consistently perceive an impression but not identify the taste. The salt taste recognition threshold was identified as the sample concentration where the participant consistently perceives the taste as salt.

2.4. Salt taste preference and self-reported salt eating habit

For the purpose of this test, tomato soup was prepared by mixing spring water (Highlands) with tomato passata (Napolina, Tesco) in 1:1 ratio. Salt (NaCl, Saxa salt) was added to manipulate the final salt concentrations of soup: 0.25%, 0.5%, 1.0%, 2.0% and 3.0% (w/w). Participants tasted each soup and rinsed their mouth with water between each sample. Saltiness and pleasantness of each of the five soups was rated on a 100 mm visual analogue scale (VAS) ranging from “not at all salty” (0 mm) to extremely salty (100 mm) and “very unpleasant” (0 mm) to “very pleasant” (100 mm). Considering that a product with salt content equal to or higher than 1.5% is considered a high salt product (British Heart Foundation, n.d.), participants that rated 2.0% and 3.0% soups as more pleasant compared to soups with 0.25%, 0.5% and 1% salt were classified as having high salt taste preference and participants that have provided the opposite ratings as having low salt preference. Self-reported salt eating habit was determined by asking participants how salty they usually believe they eat their food. Participants could answer “Eat salty”, “Eat in moderation”, “Do not eat salty” (Lee et al., 2014).

2.5. Single nucleotide polymorphism (SNP) genotyping

Genotyping was performed according to a method described elsewhere (Pilic & Mavrommatis, 2018). Pre-designed TaqMan® SNP genotyping assays for the SNPs: rs239345, rs8065080 and the StepOnePlus thermocycler (Applied Biosystems, CA, USA) with two technical replicates for each sample were used. The primers and the probes were pre-designed by Applied Biosystems with the following codes (C___2387896_30, C__11679656_10). *SCNN1B* rs239345 genotypes were not obtained for two participants. Call rates were higher than 95% and both SNPs were in Hardy Weinberg equilibrium ($p > 0.05$). *SCNN1B* rs239345 minor allele frequency (A) was 27% and the *TRPV1* rs8065080 (C) 35%, which is similar to frequencies reported in the TwinsUK database (dbSNP, 2019a; dbSNP, 2019b).

2.6. Dietary salt intake

Dietary salt intake was assessed with two 24-hour dietary recalls. It was based on the United States Department of Agriculture (USDA) 5-step multiple pass method and administered via online platform (Jisc Online Survey, Rhodes et al., 2013). The forgotten food list, in addition to the typically forgotten foods such as tea, coffee, non-alcoholic and alcoholic beverages, sweets and snacks, also contained foods usually high in salt such as pickled vegetables, deli meats, smoked fish, cheese, bread and condiments. Participants were also asked to provide information about the quantity of the stock cubes or gravy granules, if used, while cooking. Discretionary salt use was assessed asking questions on adding salt while cooking and at the table with participants providing the quantities of added salt. Energy and nutrient intake were calculated using nutritional analysis software (Nutritics, Nutritics LTD, Dublin, Ireland). Total sodium intake (non-discretionary and discretionary) was calculated as an average of sodium intake from both recalls. Additionally, it was expressed both as absolute and energy adjusted (mg sodium per 1000 kcal).

2.7. Statistical analyses

Continuous variables are presented as 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 baseline characteristics by sex were assessed

using an independent-samples t-test (with Levene's test for equality of variance), Mann Whitney U test or Fisher's exact test, as appropriate. Since previous research reported an apparent dominant mode of inheritance, major allele carriers (TT + AT for the *SCNN1B* rs239345 and TT+ CT for the *TRPV1* rs8065080) were grouped together and comparisons made against individuals homozygous for minor alleles (AA for the *SCNN1B* rs239345 and CC for the *TRPV1* rs8065080) of both SNPs (Dias et al., 2013). The associations between salt taste preference (low vs. high) and self-reported salt eating habit were tested using a Chi square test of association or Fischer's Exact test, as appropriate. A Mann Whitney U test was used to assess the difference in threshold (mmol/l) between genotypes and between participants with low and high salt taste preference. Individual ratings of saltiness and pleasantness in soup (mm) were plotted and the area under the curve (AUC) calculated using GraphPad Prism (Version 8; GraphPad Software Inc.). Difference in AUC and sodium intake (mg and mg/1000 kcal) between genotypes was tested with one-way ANOVA. Two-way ANOVA determined the interactions between thresholds and genotype group on sodium intake (mg/1000 kcal) and a three-way ANOVA included sex as an additional fixed factor. Considering there is no universal cut-off point to distinguish between the participants with low and high salt taste thresholds, a median was used as a cut-off. Participants with detection threshold ≤ 8mmol/l and recognition threshold ≤ 17mmol/l were considered to have low thresholds. Furthermore, to explore the effects of sex, two-way ANOVAs were used and sex tested for interaction with thresholds (low vs. high), preference (low vs. high) and habit with sodium intake as dependent variable (mg/1000 kcal). Analyses were conducted without and with adjustments for covariates which were age and BMI, variables often reported to be associated with taste perception and salt intake (Barragán et al., 2018; Yi, Firestone, & Beasley, 2015). Bonferroni adjustment was used for multiple comparisons. Sex-specific analyses were considered as secondary and therefore all results are not shown in results section. Analyses were performed using the SPSS software package (version 25.0). All tests were two-tailed, with $p < 0.05$ considered statistically significant.

3. Results

3.1. Participant characteristics

Characteristics of study participants are presented in Table 1. All participants were aged between 18-35 years with no difference in the mean age between males and females.

Male participants had higher BMI, detection threshold and absolute sodium intake. Overall, salt intake in the study population reflected current intakes in the UK (Department of Health, 2016). Participants were predominantly Caucasian (85%), non-smokers, professionals and highly educated (with bachelors degree or higher) and healthy (data not shown).

3.2. The genetic basis of salt taste perception and intake

The following results focus on exploring the underlying genetic basis of salt taste perception and subsequent salt intake. There was no difference in either of the thresholds or sodium intake between genotype groups of the *SCNN1B* rs239345 and *TRPV1* rs8065080 (Table 2). Sex-specific analysis revealed the same (data not shown).

Furthermore, there were no differences in the AUC for saltiness and pleasantness ratings of tomato soup between *SCNN1B* rs239345 genotype groups ($p = 0.853$ and $p = 0.636$ for saltiness and pleasantness respectively, Figure 1a and b, Table 2). Participants homozygous for the minor allele of the *TRPV1* rs8065080 had overall lower ratings of saltiness ($p = 0.008$, Figure 2a, Table 2) and higher ratings of pleasantness ($p = 0.027$, Figure 2b, Table 2) when compared to major allele carriers. Controlling for age and BMI did not affect the results. There were no differences in AUC for either of the measurements between males and females ($p = 0.268$, $p = 0.279$ for saltiness and pleasantness respectively, data not shown).

Previous research reported higher thresholds in individuals homozygous for the minor alleles of the *TRPV1* rs8065080 and *SCNN1B* rs239345, but with little reference to the actual salt intake (Dias et al., 2013). Therefore, the purpose of the following analysis was to explore if there is an interaction between genetics and threshold on energy adjusted sodium intake as the outcome variable. There was no interaction between detection threshold and any of the genotypes ($p = 0.246$ for the rs239345 and $p = 0.175$ for rs8065080 respectively). There was also no main effect of either of the variables on sodium intake (data not shown). With respect to recognition threshold, there was no interaction for the *SCNN1B* rs239345 ($p = 0.296$) or the main effect of any of the variables (Figure 3a.). However, an interaction was observed between the *TRPV1* rs8065080 and recognition threshold ($p = 0.030$). Mean sodium intake for participants with high threshold was higher in the minor allele homozygous group compared to the major allele carriers (2209 ± 376 mg/1000 kcal vs. 1323 ± 150 mg/1000 kcal, $p = 0.032$, Figure 3b.). When including sex as an additional fixed factor in the analysis,

no interaction was observed, although this may be due to very small sample size when splitting the population across the levels of three independent variables (data not shown). After adding BMI as a covariate this interaction was no longer significant (p = 0.065).

3.3. The associations between threshold, salt taste preference, self-reported salt eating habit and salt intake

The following sections will explore the associations between salt taste perception and dietary behaviour irrespective of genotype. Considering that there is clear cut-off for high salt content in food (Section 2.4), participants were dichotomised into groups that prefer lower salt soups and higher salt soups. In total population, the median detection threshold was higher in participants who preferred soups with high salt concentrations compared to those who preferred lower salt soups (0.012, interquartile range (IQR) 0.008 vs. 0.008, IQR 0.006, p = 0.029). There was no difference in recognition threshold between the preference groups (p=0.413). There were no sex-specific differences observed (data not shown). Additionally, no interactions were observed between sex and detection (p = 0.230) or recognition threshold (p = 0.561) on sodium intake (mg/1000 kcal). There were also no main effects of sex or thresholds on sodium intake (mg/1000 kcal). Controlling for age and BMI did not affect the results (data not shown).

Furthermore, there was an association between preference for salt in soup and self-reported salt eating habit where a larger proportion of participants preferring high salt soup was in the “Eat salty” compared to the “Eat in moderation” or “Do not eat salty” sub-groups (50% vs. 8% for the latter two groups, p = 0.003, Figure 4a). When stratifying according to sex, this association was seen only in females (p = 0.003). Two-way ANOVA revealed that there was no interaction between sex and salt taste preference on sodium intake (p = 0.246), however, participants who were classified as having high salt preference had higher sodium intake compared to those who had a low salt preference (2236 ± 261 vs. 1543 ± 107 mg/1000 kcal, p = 0.017). In addition, participants who reported to eat salty food had higher mean sodium intake compared to participants who reported they do not eat salty (2487 ± 274 vs. 1383 ± 94, p = 0.007, Figure 4b). There was no interaction between habit and sex on sodium intake (p = 0.070). Controlling for age and BMI did not affect the results.

4. Discussion

The aim of the present study was to explore the associations between genetics, salt taste perception (threshold sensitivity), preference and habitual dietary intake of salt in young and healthy adults. We hypothesised that genetic variants (rs239345 and rs8065080) in two putative salt taste receptors (ENaC and TRPV1), previously associated with salt taste sensitivity, will determine salt taste perception and preference for salt in a food product. Furthermore, we hypothesised that preference would drive salt habit and the actual salt intake.

4.1. Genetics of salt taste perception

We found no direct association between genetics and salt taste detection and recognition thresholds, irrespective of sex. Although Dias et al. (2013) reported lower suprathreshold salt taste sensitivity in participants homozygous for the minor allele of the *SCNN1B* rs239345 (AA genotype) and *TRPV1* 8065080 (CC genotype), they also did not observe an association with detection thresholds in a population of young and healthy adults. Conversely, a more recent study conducted in a large European cohort suggested that the AA group of the rs239345 perceived salty taste more intense than the major allele carriers (Barragán et al., 2018). However, this association was weak and the effect of this SNP on detection threshold may not be large enough to be detected in a smaller sample size study. Contradictory results may be explained by different methods of measuring taste perception between the above-mentioned studies, including the present. Additionally, the age ranges, which differ between studies, together with differences in study populations may explain the discrepancies in results. The fact that no association between the two SNPs and thresholds was observed in the present study does not necessarily mean *SCNN1B* and *TRPV1* have no effect on salt taste thresholds. It may be that other SNPs, not investigated in the present study, have a more pronounced role. Although measuring suprathreshold sensitivity, Chamoun et al. (2018) suggested that the *TRPV1* rs161386, rs222745 and rs150908 play a role in salt taste perception in healthy, younger to middle-aged Canadian adults and these variations warrant further investigation.

As stated in the introduction, the *TRPV1* rs8065080 is functional, with minor allele C associated with lower protein activity (Cantero-Recasens et al., 2010). This may explain why participants with CC genotype were reported to have higher thresholds (Dias et al., 2013).

Indeed, we observed that participants homozygous for the minor allele of the *TRPV1* rs8065080 perceived tomato soups as less salty compared to major allele carriers. It should be noted that salt concentrations used in tomato soups were higher than the concentrations used to test for thresholds. These reflected salt content in food products, ranging from low to high, which may be more representative of the actual acceptance of salt in food compared to tests using water. In this sense, Chamoun et al. (2018) reported on the association between the *TRPV1* rs150908 and preference for salty taste in hummus, which suggests that this receptor may be involved in hedonic response to food. In the present study, in addition to having lower ratings of saltiness, *TRPV1* rs8065080 minor allele homozygous participants also perceived the soups as more pleasant. Regarding the *SCNN1B* rs239345, no significant associations may be due to small sample size in the group homozygous for the minor allele and results warrant further investigation.

However, even if the involvement of rs8065080 in salt taste perception is authentic, it is important to explore if genetics and/or salt taste perception influence actual salt intake. To the best of our knowledge, research to date does not explore this in context of salt intake in adults, whereas it was suggested that SNPs in *TRPV1* were not associated with salt intake in children (Chamoun et al., 2018). In the present study, the potential effect of genetics on salt intake was apparent in participants with high thresholds. Participants homozygous for the minor allele of the *TRPV1* rs8065080 had higher sodium intake compared to the major allele carriers, after controlling for age. Sex did not seem to play a role, although these interactions should be explored in a larger sample size study. Finally, the interaction between genetics and threshold was no longer significant ($p = 0.065$) after controlling for BMI which may imply that this variable is more strongly associated with sodium intake than genetics. However, BMI was not associated with sodium intake, thresholds or genetics in this population so it is difficult to pinpoint the reasons for the latter observation. Nevertheless, this study, for the first time, suggests that *TRPV1* rs8065080 may have a role in salt intake. Considering an inconclusive link with BMI and a relatively small sample size in sub-group analyses, these results may be considered as hypothesis-generating and require replication. Ideally, future studies should have a sample large enough to be able to explore the effects of genetics (both *SCNN1B* and *TRPV1*) on salt intake in a covariate-dependent manner, primarily stratifying the population according to sex, age and BMI categories.

Nevertheless, it may be that when rs8065080 minor allele carriers have higher threshold, salt intake is higher compared to major allele carriers because of a more

pronounced hedonic response. This information may in the future be used to inform more personalised dietary interventions. Rankin et al. (2018) suggest that sensory appeal is one of the most important factors of food choice in their large pan European study and highlight the need to account for sensory preferences when providing personalised nutrition services.

4.2. The associations between salt taste preference, self-reported salt habit and salt intake

As suggested above, sensory appeal is for many consumers more important than health in making food choice decisions (Rankin et al., 2018). However, research is conflicting regarding the link between threshold, preference and intake, possibly due to differences in methods and populations studied. Moreover, it often does not consider all variables comprehensively.

Indeed, the associations between taste thresholds and preference for a specific taste are controversial in the literature. While some studies reported on an inverse association between these two variables, both in older and younger adults (Barragán et al., 2018; Chamoun et al. 2019), other studies showed the opposite (Bossola et al., 2007). Our results suggest that participants who rated high salt soups as more pleasant may have higher salt taste detection threshold, however due to small sample size in this sub-group analysis and the method of measuring detection threshold, results may be considered as preliminary.

Nevertheless, it is hypothesised that individuals with increased taste sensitivity require a lower concentration of a specific stimulus and when that concentration is perceived as high, a negative hedonic response is elicited. It may be expected that there is a direct association between taste sensitivity and salt intake (i.e. high taste sensitivity leading to a lower intake), however, research is conflicting. Fischer et al. (2012) report on an inverse association between salt taste intensity, measured with a filter paper disk impregnated with 1.0 mol/l sodium chloride, and the frequency of discretionary salt use in their population of middle-aged adults. Contrary to this, salt taste perception was not related to sodium consumption, assessed with one 24-hour recall and 14 consecutive food records, in a sample of 24 young adults aged 20 to 30 years (Drewnowski, Henderson, Driscoll, & Rolls, 1996). Similarly, we found no direct association between thresholds and energy adjusted sodium intake irrespective of sex, age or BMI. However, participants who were classified as having high salt preference had higher sodium intake compared to those rating low salt soups as

more pleasant. In this sense, taste preference (hedonic component) may serve as a "bridge" between a more physiological aspect of taste perception such as taste threshold and a dietary behaviour- salt intake.

Other studies also reported that individuals who preferred higher concentrations of salt in tomato soup had higher salt intake (Hayes, Sullivan, & Duffy, 2010). It should be noted however, that preference may be more strongly associated with discretionary than non-discretionary salt use (Hayes, Sullivan & Duffy, 2010). For example, Takachi, Ishihara, Iwasaki, Ishii, & Tsugane (2014) highlighted that the self-reported taste preference for miso soup was associated with total daily sodium consumption in middle-aged Japanese adults. The authors also showed that discretionary salt-related behaviour in association with taste preference may be a defining factor of daily salt intake. Although this is the case in populations where discretionary salt use accounts for the majority of salt intake, preference for salty taste may explain a proportion of salt intake even in populations where non-discretionary salt accounts for approximately 75% of the daily salt (Brown, Tzoulaki, Candeias, & Elliott, 2009). Literature also suggests that salt habit may be used as a proxy to establish salt taste preference in Korean adults (Lee et al., 2014). Although in a different population, we observed an association between preference and habit. This appeared to be the case only in females. Considering a lower number of males in the present study it may also be the case of insufficient power to detect the same in this group and therefore, sex specific analyses should be considered as preliminary. Nevertheless, Hayes et al. (2010) report how healthy females have higher preference for saltier foods than males, highlighting the importance of considering sex differences in salt taste preference and consumption. Furthermore, similar to what was reported previously (Lee et al. 2014) and was hypothesised in this study, self-reported salt eating habit did translate into the actual amount of salt consumed and may potentially be used as a proxy to determine salt consumption if further developed into a questionnaire. For example, D'Elia, Manfredi, Strazzullo, & Galletti (2019) developed a short questionnaire on the assessment of salt habit in hypertensive patients that reflects their salt intake. Based on the results of the present study, a similar approach may be employed in a younger, healthy population.

Finally, even if the above reported associations are more reflective of discretionary salt intake, reduction of salt content in processed food may result in the actual increase in discretionary salt use (Quader et al., 2016). Therefore, a better understanding of this behaviour may enable more targeted public health interventions to reduce salt intake.

4.3. Strengths and limitations

A strength of this study is the use of two 24-hour dietary recalls. By using more than one 24-hour recall, accuracy of total sodium intake measurement increases (Freedman et al., 2015). This recall was based on the USDA automated multiple pass method (AMPM) recall which is suggested as a valid method for assessing dietary salt intake (McLean, 2014; Rhodes et al., 2013). Nevertheless, this is the case for the US adult population and further validation studies are needed to assess its accuracy in a population similar to this one. Although there may be a case of misreporting, sodium intake was energy adjusted, which also improves accuracy (Freedman et al., 2015). Furthermore, discretionary salt intake was quantified in the present study, which was not the case with AMPM (Rhodes et al., 2013). Therefore, this 24-hour recall may capture total salt intake more accurately. Indeed, salt intake reflected the intakes reported in the UK adult population (Department of Health, 2016). The use of a tomato soup as a vehicle may have introduced "noise" in participant perception of salt due to interactions with other flavours present in this food alongside other organoleptic properties of tomato soup. However, utilising an actual food instead of water and with salt concentrations similar to food products, may be more realistic and applied to food preference and choice. Although only 74 participants completed the taste preference test, which may be considered a limitation, this sample size is similar to a sample of adults in a recent study exploring the associations between genetics and taste preference (Chamoun et al., 2018). Furthermore, dichotomising participants into those who prefer low vs. high salt soup may not be the most accurate as salt concentrations in soup reflected food products with low, medium and high salt content. Future studies should include a further low salt soup concentration to be able to categorise participants in three respective groups of preference. Additionally, taste sensitivity and preference measures should be repeated on multiple occasions to ensure further validity. Finally, a smaller proportion of participants was classified as having high salt preference and reported to eat salty food which may have affected the results. Nonetheless, as suggested above, salt intake in this study did represent intakes in the UK population implying that the dietary behaviour of this study population may reflect the behaviour of a wider population of similar demographic characteristics to this one.

5. Conclusion

The results of the present study suggest that genetic variations play a role in salt taste perception with the *TRPV1* rs8065080, for the first time, suggested as the variant not only

affecting perception of salt in water but also perception of salt in a food product. Although considered as a hypothesis-generating result, it appears that this variant also plays a role in salt intake. If this is confirmed, intervention studies exploring possibilities to enhance perception of salty taste in individuals homozygous for the minor allele of this SNP are warranted. Preference for salty taste and self-reported salt eating habit are correlated and both associated with total salt intake in this population. Therefore, a hedonic appeal of salty food should be considered when providing personalised nutrition advice aimed at changing this behaviour in a population similar to this one.

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Declaration of Interest

Dr. Yiannis Mavrommatis is a shareholder for Nell Health, a lifestyle genotyping company.

Author contributions

Leta Pilic: Conceptualization**,** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualisation **Nicole Jane Lubasinski**: Investigation, Data Curation **Melis Berk**: Investigation, Data Curation **Delia Ward**: Investigation, Data Curation **Catherine Anna-Marie Graham**: Writing – Review and Editing **Viviane Da Silva Anastacio**: Writing – Review and Editing **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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Tables

Table 1. Baseline characteristics of study participants (n = 95). Data presented as mean ± SEM or absolute (relative) frequencies. P value for difference between male and female participants (Independent samples t-test, Mann Whitney-U test, Fischer's Exact test).

	Male (n = 32)	**Female (n = 63)**	**p**
Age (years)	29.6 ± 1.1	26.6 ± 0.9	0.058
BMI (kg/m^2)	25.1 ± 0.5	23.1 ± 0.5	0.010
STDT (mmol/l)[a)]	12 (13)	8 (6)	0.029
STRT (mmol/l)[a)]	17 (12)	12 (5)	0.328
Preference for salt in soup (n = 74)			
Low	22 (78.6)	43 (91.5)	0.161
High	6 (21.4)	4 (8.5)	
Self-reported salt habit (n = 74)			
Do not eat salty	16 (59.3)	24 (51.1)	0.839
Eat in moderation	8 (29.6)	16 (34)	
Eat salty	3 (11.1)	7 (14.9)	
Sodium intake (mg)	3358 ± 299	2878 ± 284	0.020
Salt intake (g)	8.4 ± 0.7	7.2 ± 0.7	
Sodium intake (mg/1000 kcal)	1642 ± 172	1731 ± 142	0.192

a) median (interquartile range); body mass index (BMI), salt taste detection threshold (STDT), salt taste recognition threshold (STRT)

Table 2. Key outcome variables according to the *SCNN1B* rs239345 and *TRPV1* rs805080 genotype**.** Data presented as mean ± SEM or median (interquartile range). P value for difference between genotype groups (One-way ANOVA, Mann Whitney-U test).

	rs239345			rs8065080		
	TT+AT (n=87)	AA (n=6)	p	TT+ CT (n=81)	CC (n=14)	p
STDT (mmol/l)	8 (6)	8 (11)	0.383	8 (6)	8 (8)	0.506
STRT (mmol/l)	17 (12)	14.5 (13)	0.943	17 (12)	12 (16)	0.295
AUC saltiness*	194 ± 5	198 ± 31	0.853	200 ± 5	161 ± 15	0.008
AUC pleasantness*	91 ± 7	94 ± 12	0.636	85 ± 7	123 ± 10	0.027
Sodium intake (mg/1000 kcal)	1630 ± 83	1715 ± 411	0.890	1622 ± 84	1719 ± 274	0.853
Sodium intake (mg)	3047 ± 166	3276 ± 717	0.672	3060 ±165	3136 ± 489	0.832

* Sample size: rs239345 TT+AT (n=70) and AA (n=4); rs8065080 TT+ CT (n=62), CC (n=12); area under the curve (AUC), salt taste detection threshold (STDT), salt taste recognition threshold (STRT).

Figure legends

Figure 1. Mean saltiness and pleasantness ratings of tomato soup according to *SCNN1B* rs239345 genotype. Error bars represent ± SEM. Area under the curve difference between genotypes (p = 0.853 for saltiness and p = 0.636 for pleasantness respectively, One-way ANOVA).

Figure 2. Mean saltiness and pleasantness ratings of tomato soup according to *TRPV1* rs8065080 genotype. Error bars represent ± SEM. Area under the curve difference between genotypes (p = 0.008 for saltiness and p = 0.027 for pleasantness respectively, One-way ANOVA).

Figure 3. Mean sodium intake (mg/1000 kcal) across recognition threshold and *SCNN1B* rs239345 (a) and *TRPV1* rs8065080 (b) genotype groups. Error bars represent ± SEM. Two-way ANOVA (Bonferroni adjusted p values; p for interaction in figure b = 0.030).

Figure 4. Self-reported salt eating habit in context of preference for salt in soup (a) and the mean sodium intake (mg/1000 kcal) (b). Error bars represent ± SEM. Fischer's Exact test (a) and one-way ANOVA (b) (Bonferroni adjusted p value).

Figure 1.

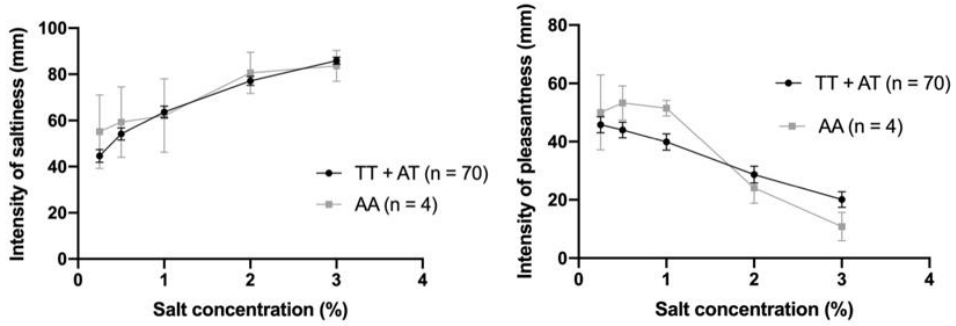

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Figure 2.

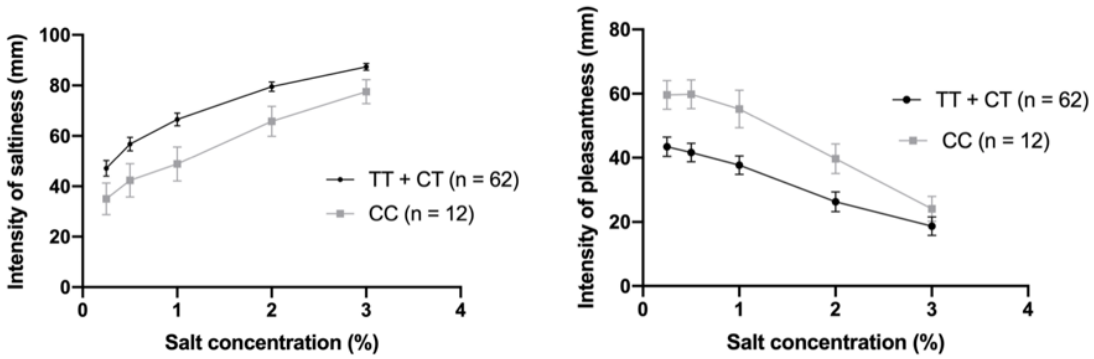

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Figure 3.

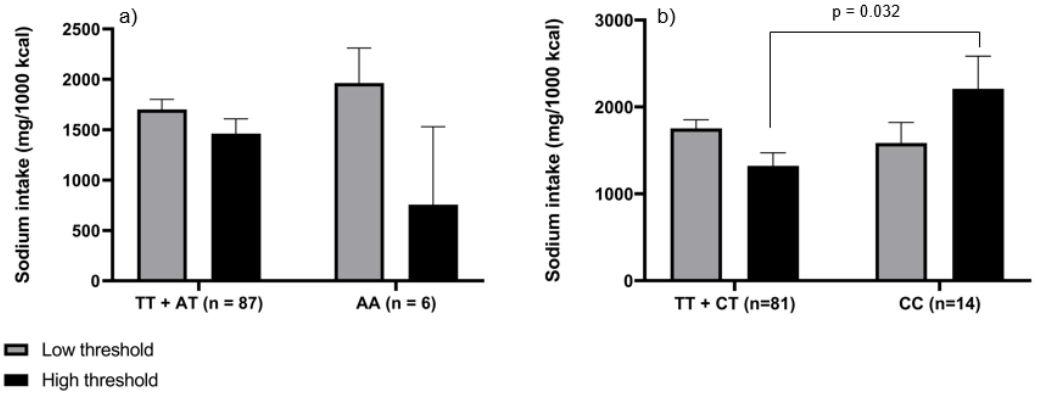

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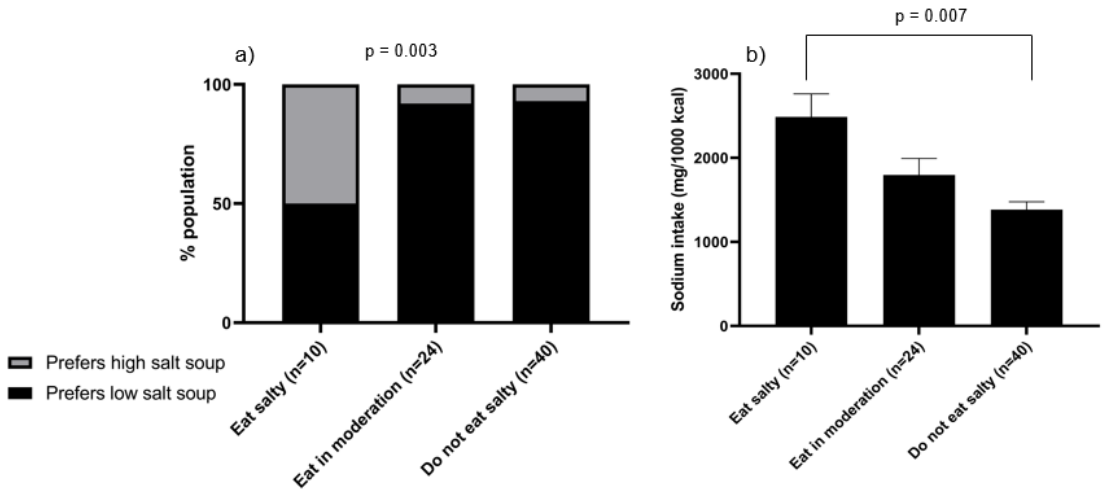
Figure 4


a)
p = 0.003
100
50
0
% population
Prefers high salt soup
Prefers low salt soup
Eat salty (n=10)
Eat in moderation (n=24)
Do not eat salty (n=40)
b)
p = 0.007
3000
2000
1000
0
Sodium intake (mg/1000 kcal)
Eat salty (n=10)
Eat in moderation (n=24)
Do not eat salty (n=40)

666