Genetic predisposition to salt-sensitive normotension and its effects on salt taste perception and intake (Genetics of salt sensitivity and salt intake)

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ABSTRACT

Salt sensitivity is an independent CVD and mortality risk factor, present in both hypertensive and normotensive populations. It is genetically determined and it may affect the relationship between salt taste perception and salt intake. The aim of this study was to explore the genetic predisposition to salt sensitivity in young and a middle-aged adult population and its effects on salt taste perception and salt intake. The effects of sodium loading on blood pressure (BP) were investigated in 20 normotensive subjects and salt sensitivity defined as the change in BP after seven days of low sodium (51.3 mmol sodium/day) and seven days of high sodium diet (307.8 mmol sodium/day). Salt taste perception was identified using the British Standards Institution sensory analysis method (BS ISO 3972:2011). Salt intake was assessed with a validated FFQ. DNA was genotyped for SNPs in the *SLC4A5*, *SCNN1B* and *TRPV1* genes. The subjects with AA genotype of the *SLC4A5* rs7571842 exhibited the highest increase in BP (ΔSBP=7.75 mmHg, p=0.002, d=2.4; ΔDBP=6.25 mmHg, p=0.044, d=1.3; ΔMAP=6.5 mmHg, p=0.014, d=1.7). The *SLC4A5* rs10177833 was associated with salt intake (p=0.037) and there was an association between salt taste perception and salt sensitivity (r_s=0.551, p=0.041). The association between salt taste perception and discretionary salt use may depend on the *SLC4A5* and *TRPV1* genotype. In conclusion, there is a genetic predisposition to salt sensitivity and it is associated with salt taste perception. The association between salt taste perception and discretionary salt use suggests that preference for salty taste may be a driver of salt intake in healthy population and warrants further investigation.

Keywords: blood pressure, genetics, salt sensitivity, salt intake, taste

1. Introduction

Hypertension is a major cause of CVD and overall mortality [1]. High dietary sodium intake is a major risk factor for hypertension [2,3] estimated to be responsible for one in 10 deaths from CVD events [4]. In 2010, the estimated mean global sodium consumption was 3.95 g per day, with regional mean levels ranging from 2.18 g to 5.51 g/day, exceeding the reference intake of 2.0 g of sodium/day [4,5].

One of the main determinants of food intake, and potentially salt, is taste [6]. The ability to perceive a certain taste may be genetically determined [7]. More specifically, genetic variation in taste receptors may alter an individual's taste function [8]. However, to our knowledge, only one study reports the genetic predisposition to salt taste in humans. SNPs in genes coding for ion channels, the epithelial sodium channel (*SCNN1B*) rs239345 and the transient receptor potential cation subfamily V member 1 channel (*TRPV1*) rs8065080, modified the salt taste perception in 95 white young adults [8]. The effect of these genetic variants on actual sodium intake has not been investigated and the results warrant further investigation. In addition, a link between salt taste perception and blood pressure (BP) is suggested. A number of studies reported that individuals with lower ability to taste salt (i.e. reduced salt taste sensitivity) exhibited higher BP compared to individuals with enhanced ability to perceive salty taste. This was observed both in adults and children and across different populations [9-12]. Moreover, research suggests an association between salt taste sensitivity and salt intake, albeit inconclusive [13,14]. Considering the above and with the notion that high salt intake is a major risk factor for raised BP [2,3], it can be hypothesised that reduced salt taste sensitivity would result in higher dietary salt intake and consequently in higher BP.

Furthermore, the mechanisms behind the possible link between salt taste perception and salt intake are unclear and confounded by other metabolic and physiological aspects of salt metabolism. The main confounder is salt sensitivity which is defined as an increase in BP in response to a high dietary salt intake [15]. Considering that some individuals do not exhibit such increase, the distinction is made between salt-sensitive and salt-resistant populations [16]. Salt sensitivity displays a strong heritable component and the genes involved in sodium transport across the cell membrane have shown a strong effect on salt-sensitive changes in BP [17,18]. Specifically, rs7571842 and rs10177833 in the *SLC4A5* gene, coding for electrogenic sodium bicarbonate cotransporter 2, have been associated with salt sensitivity in Caucasian hypertensive and normotensive populations [19]. In addition to salt taste perception, the *TRPV1* gene has been associated with salt sensitivity in

animals[20,21]. Wang and Wang[20] have reported that in Dahl salt-sensitive rats on a high-salt diet, TRPV1 expression and function is impaired rendering these rats sensitive to salt load in terms of BP regulation. Furthermore, the *TRPV1* rs8065080 is a missense SNP resulting in amino acid change at position 585, from isoleucine to valine, potentially affecting protein function [22]. Cantero-Recasens et al. [23] have tested its functional effect by expressing it in HeLa cells and showed a decreased channel activity in response to two typical TRPV1 stimuli, heat and capsaicin, in TRPV1-Val-585 cells compared to TRPV1-Ile-585. The loss of function effect of the rs8065080, together with reduced expression and activity of the TRPV1 reported in salt-sensitive animals suggests this variant may also be involved in salt sensitivity in humans. Finally, several common variants of the epithelial sodium channel *SCNN1B* gene, including the rs239345, have been associated with BP or hypertension in different populations [24,25].

Recent research in animals suggests an association between salt taste perception and salt-sensitive hypertension mediated by the renin-angiotensin aldosterone system (RAAS) dysfunction [26]. To the best of our knowledge, there are no studies in humans confirming this association. In addition, there are no studies comprehensively exploring the link between salt sensitivity of BP, salt taste perception and intake. Furthermore, salt sensitivity is present in 51% of hypertensive and 26% of normotensive populations and it is an independent cardiovascular and mortality risk factor [27,28]. Since reduction in salt intake may lead to significant reductions in BP in susceptible individuals [1,2], detecting salt sensitivity in young and healthy individuals may result in more successful prevention of hypertension and consequently CVD [29].

Considering the potential link between salt sensitivity, salt taste perception and dietary salt intake together with the underlying genetic basis, the aim of this study was to explore the genetic predisposition to salt sensitivity, expressed as the BP response to sodium loading, in a healthy adult population and its effects on salt taste perception and dietary salt intake.

2. Methods

2.1. Subjects

The subjects were predominantly young Caucasians, eight males and 12 females. Subjects were recruited through advertisements, internet postings and the institutional Centre for Workplace and Community Health. Eligibility criteria were clearly stated. More specifically, subjects were excluded with current stage-2 hypertension (systolic blood pressure (SBP) ≥160 mm Hg and/or

diastolic blood pressure (DBP) ≥100 mm Hg), current or recent (less than one month prior to screening visit) use of anti-hypertensive medications or medications that affect BP. Further, those with secondary hypertension, history of CVD, chronic kidney failure, current diabetes were excluded. Also excluded were individuals with peptic ulcer disease or liver disease requiring treatment during the previous two years. In addition, pregnant women, underweight (BMI <18.5 kg/m2) and obese (BMI>30 kg/m2) individuals, individuals exceeding maximal recommended alcohol intake for the UK, those currently adhering to a low sodium diet, or with an illness that permanently alters taste were also excluded from the study.

All 20 subjects completed the taste threshold determination test to assess salt taste perception, FFQ and provided a saliva sample. Out of 20 subjects, 19 completed the low- and high sodium dietary protocols, however, five subjects were excluded due to incomplete 24-hour BP or urinary excretion data (Figure 1).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Ethics Committee. Written informed consent was obtained from each subject before the baseline data collection informing they can withdraw from the study at any point. The study is registered under Research Registry unique identification number: researchregistry1652.

2.2. Baseline measurements

Height and baseline BP and weight were measured during the first examination. Subjects were instructed to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their BP measurement. Seated BP was measured with an automated BP monitor (OMRON M24/7, Milton Keynes) using an appropriate size cuff after five minutes of rest. Two measurements were performed within five minute intervals and used for the analysis and calculation of the mean baseline SBP and DBP. In addition, demographic data (age, sex and race) was collected and assessed together with smoking habits and health status information. Physical activity was assessed with the General Practice Physical Activity Questionnaire. Participants were considered as: active, moderately active, moderately inactive or inactive [30].

123 **2.3. Taste thresholds for salt**

124 Identification of taste thresholds for salt (salt taste perception) was determined using the
125 British Standard BS ISO3972:2011 methodology. Salt taste detection and recognition thresholds
126 were determined using eight graded sodium chloride solutions (4 mmol/l, 6 mmol/l, 8 mmol/l, 12
127 mmol/l, 17 mmol/l, 24 mmol/l, 34 mmol/l and 49 mmol/l). Solutions were prepared by dissolving
128 food grade sodium chloride in spring water. All solutions were prepared on the day of the testing.
129 Subjects were presented with a sample of each solution by order of increasing concentration starting
130 with the lowest concentration of 4 mmol/l. The procedure was repeated three times. Three
131 additional vessels containing dilutions of the same concentration as the preceding vessel were
132 presented randomly within the sample series. The salt taste detection threshold (STDT) was
133 identified as the lowest concentration of the sample where the subject can consistently perceive an
134 impression but not identify the taste. The salt taste recognition threshold (STRT) was identified as
135 the sample concentration where the subject consistently perceives the taste as salt [(31)].

136

137 **2.4. Habitual dietary salt intake**

138 Baseline energy and dietary salt intake were assessed using a semi-structured validated FFQ.
139 The questionnaires were analysed using the open source, cross-platform tool FETA [(32)] and
140 information on 46 nutrients, including sodium, was obtained. Habitual dietary sodium intake was
141 energy adjusted and expressed as mg of sodium per 1000 kcal. Information on the frequency of
142 discretionary salt use was also obtained. Subjects recorded the frequency of adding salt while
143 cooking and at the table by choosing one of the following: 1) never, 2) rarely, 3) sometimes, 4)
144 usually and 5) always.

145

146 **2.5. Dietary sodium intervention**

147 Study subjects received a low-sodium diet (3 grams of salt or 51.3 mmol of sodium/day) for
148 seven days, followed by a high-sodium diet (18 grams of salt or 307.8 mmol of sodium/day) for an
149 additional seven days. Minimal wash-out period between the diets was seven days. The low sodium
150 diet was designed by investigators using the nutritional analysis software (Nutritics, Nutritics LTD,
151 Dublin, Ireland). Three meals and two snacks were designed to provide a total of 3 grams of salt per
152 day and recommended macronutrient intake [(33)]. Total energy intake was determined based on
153 individual requirements of each subject. Subjects were provided with detailed written instructions

about the diets and they were also instructed to maintain their coffee, smoking and physical activity levels. The high sodium diet was formulated by supplementing the low-sodium diet with additional 256.5 mmol of sodium/day (15g of salt per day) dispensed by research staff in small paper sachets each containing 1g salt (NaCl). To monitor subject compliance with the diets, on the last day of each period, 24-hour urine was collected for sodium, potassium and creatinine excretion measurements. During the same period, 24-hour BP measurements were performed with the 24-hour ambulatory BP monitoring device (ABPM).

2.6. Twenty-four-hour automated BP monitoring

Twenty-four–hour ABPM was attached to the upper, non-dominant arm and BP was registered at 30-minute intervals during daytime and 60-minute intervals at night time. Data from the ABPM was downloaded using BP Tracker Software and mean SBP and DBP were calculated. Subject data with less than 30 successful measurements on each occasion was excluded from the analysis for salt sensitivity [34]. Pulse pressure (PP) was calculated according to the formula: PP = SBP – DBP and mean arterial pressure (MAP) as: MAP = DBP + 1/3 PP. Salt sensitivity was defined as an increase of ≥ 3 mmHg in MAP when transitioning from the low to high sodium diet, as suggested by Kurtz et al. [35]. The change in BP between the high sodium and low sodium diet (ΔBP) was calculated as: ΔBP = high sodium diet BP – low sodium diet BP.

2.7. Biochemical measurements

The 24-hour urinary sodium and potassium were analysed using an automated clinical chemistry analyser (Randox: Rx Daytona), with intra-assay CV < 6%. Estimated salt intake was calculated using the equation 17.1 mmol of sodium = 1g of salt. Assessment of the completeness of the collection was assessed by measuring creatinine levels from the same urine samples. The following criteria were used: 1) incomplete urine = <0.7 of [mmol urinary creatinine x 113]/[21 x kilograms of body weight] [36], 2) urinary creatinine <4 mmol/day for women, or <6 mmol/day for men, or a 24 h urine collection of <500 mL for either sex and extreme outliers for urinary creatinine (ie, >3 SD from the mean) considered as unacceptable [37]. Subjects with incomplete urine collection from any of the dietary intervention periods, based on any of the two criteria, were excluded from the analysis.

2.8. Single nucleotide polymorphism (SNP) determination

Following the extensive literature review, four SNPs were selected for genotyping: rs7571842 (A/G) and rs10177833 (A/C) in the *SLC4A5* gene, rs239345 (T/A) in the *SCNN1B* and rs8065080 (T/C) in the *TRPV1* gene. These SNPs were chosen based on their previously reported associations with BP phenotypes, such as hypertension or salt sensitivity, and salt taste perception. This was combined with prevalence data (minor allele frequencies) for the SNP [8,19,38] (Supplementary Table 1).

At baseline examination a 2 ml saliva sample was collected into a collection vial (SalivaGene collection module II, STRATEC Molecular, Berlin). A stabiliser provided by the manufacturer was added to the saliva sample and it was stored at -20 °C until DNA was extracted. Genomic DNA was extracted using a commercial kit PSP® SalivaGene 17 DNA Kit 1011 (STRATEC Molecular, Berlin) in accordance with the manufacturer protocol. Quality and quantity were assessed using Nanodrop (ThermoFisher, Waltham, MA, USA). Genotyping was performed using a pre-designed TaqMan® SNP genotyping assays for the SNPs: rs7571842, rs10177833, rs239345, rs8065080 and the StepOnePlus thermocycler (Applied Biosystems, CA, USA) with two technical replicates for each sample. The primers and the probes were pre-designed by Applied Biosystems with the following codes (C____197439_10, C___1137534_10, C___2387896_30, C__11679656_10). The PCR amplification was performed under the conditions specified by the manufacturer. SNPs were accepted when the quality threshold was above 98%. All SNPs had minor allele frequencies higher than or equal to 30% and these reflected the ones reported in European populations [38] (Supplementary Table 2).

2.9. Statistical analysis

Sample size calculation was based on the 4 mmHg difference in MAP when transitioning from low to high sodium diet. This difference in BP was observed in other studies investigating salt sensitivity in normotensive populations and with a 24-hour ABPM [39,40]. A sample size of 15 was calculated using an alpha of 0.05, power of 80%, expected large effect size (d=0.8) and a standard deviation of 5 mmHg. This standard deviation was chosen due to lower variability of BP reported in younger and healthy individuals [40,41].

All continuous variables are presented as mean and SEM or median (interquartile range). Categorical variables are presented as absolute (relative) frequencies. Before further statistical

analysis, continuous variables were tested for normality with the Shapiro-Wilk test. Differences in baseline characteristics by salt sensitivity status were assessed using an independent samples t-test (with Levene's test for equality of variance) or Fischer's exact test. The difference between clinical characteristics of subjects between the low and high sodium diets was assessed using paired samples t-test. An independent samples t-test (with Levene's test for equality of variance) or Mann-Whitney U test, as appropriate, was used to test for the difference in salt-sensitive changes in BP and dietary sodium intake by genotypes of interest. The model used for the analysis was: major allele homozygote versus heterozygote plus minor allele homozygote. A Cochran Armitage test of trend was run to determine whether a linear trend exists between the genotypes of interest and the proportion of subjects with low and high STDT and STRT as well as the proportion of subjects in different tertiles of energy adjusted sodium intake. Considering there is no universal cut-off point provided to distinguish between the subjects with low and high salt taste thresholds, a median was used as a cut-off. Subjects with STDT $\leq$ 8 mmol/l and STRT $\leq$12 mmol/l were considered to have low thresholds.

To assess the relationship between salt taste thresholds and salt-sensitive changes in BP and salt taste thresholds and sodium intake, Spearman's correlation analysis was performed. Analyses were performed using the SPSS software package (version 22.0, Chicago, IL, USA). All tests were two-tailed, with $p < 0.05$ considered statistically significant.

3. Results

3.1. Subject characteristics and compliance with the dietary sodium intervention

Twenty subjects completed the baseline examination, taste threshold determination test and FFQ. Of these, 14 subjects provided complete 24-hour ABPM and 24-hour urine excretion data and were included in the analysis on salt sensitivity of BP. Five subjects were considered salt-sensitive using the criteria of $\geq$ 3 mmHg increase in MAP when transitioning from low to high sodium diet. The study population was normotensive, predominantly white, physically active and non-smoking with a median age of 28 years (Table 1). There was no significant difference in any of the baseline parameters between salt-sensitive and salt-resistant subjects.

In addition, there was no difference in BP between the low sodium and high sodium diet periods (Table 2). Urinary sodium excretion results demonstrated good compliance with the diet ($p<0.0005$) whereas potassium intake remained similar on both diets ($p=0.243$).

3.2. Genetic predisposition to salt sensitivity of BP, altered salt taste perception and salt intake

Regarding the genetic predisposition to salt sensitivity, the mean change in BP between the low and high sodium diet differed according to *SLC4A5* rs7571842 genotype (Figure 2). The subjects with AA genotype had the highest increase in BP (ΔSBP=7.75 ± 1.44 mmHg, p=0.002, d=2.4; ΔDBP=6.25 ± 2.81 mmHg, p=0.044, d=1.3; ΔMAP=6.5 ± 2.10 mmHg, p=0.014, d=1.7). SNPs rs10177833 (*SLC4A5*) (Figure 2), rs239345 (*SCNN1B*) and rs8065080 (*TRPV1*) had no statistically significant effects on the BP response to dietary sodium manipulation (data not shown). Moreover, the analysis was conducted to test for the possible difference in the prevalence of males and females, BMI and age, between the rs7571842 genotype groups. There was no difference in any of the variables between the AA and AG + GG group (p=1.000, p=0.846 and p=0.584 for sex, BMI and age respectively).

In contrast with the above described, the proportion of study subjects with low and high salt taste recognition thresholds was similar according to genotypes of interest (Figure 3). The results of a Cochrane Armitage test of trend between the different genotype groups (homozygous major allele, heterozygous and homozygous minor allele) and the proportion of subjects with low and high STRT were: rs7571842 (p=0.905), rs10177833 (p=0.714), rs239345 (p=0.456), rs8065080 (p=0.078). Similar were observed for STDT (data not shown). However, a linear trend was observed regarding the distribution of subjects in the first or second + third tertile of energy adjusted sodium intake according to the *SLC4A5* rs10177833. With the increasing number of A alleles, sodium intake increased (p=0.037, Figure 4). The mean age and BMI as well as the distribution of sex did not differ between the rs10177833 genotype groups (p=0.129, p=0.551, p=1.000 for age, BMI and sex respectively).

3.3. Associations between salt sensitivity of BP, salt taste perception and salt intake

When exploring the associations between the main outcome variables, there was no correlation between the mean change in SBP, DBP and MAP, when transitioning from a low to high sodium diet, and salt taste thresholds (Table 3). However, a positive moderate correlation was observed between the mean change in PP and STDT (r_s=0.551, p=0.041). Sub-group analysis revealed a strong positive correlation between the change in PP and STDT in the *SLC4A5* rs7571842 AG + GG group (r_s=0.845, p=0.002). Similar was observed for the rs10177833. There

was a strong positive correlation between the change in PP and STDT in the AC + CC group (rs=0.781, p=0.022, Supplementary Table 3).

Furthermore, in the total study population, the correlation between the STDT and energy-adjusted sodium intake was not significant (r_s=0.069, p=0.774). Similar was observed for STRT (r_s=0.025, p=0.918). In addition, the correlation between adding salt while cooking and at the table and salt taste thresholds was also investigated. No significant correlation was observed (STDT: r_s=0.134, p=0.573 for adding salt at the table and r_s=0.342, p=0.140 for adding salt while cooking; STRT: r_s=0.083, p=0.727 for adding salt at the table and r_s= - 0.071, p=0.767 for adding salt while cooking, Supplementary Table 4). However, as shown in Figure 5, when stratifying according to genotype, in the AA group of the *SLC4A5* rs7571842, a strong and positive correlation was observed between adding salt while cooking and both STDT (r_s=0.868, p=0.011) and STRT (r_s=0.868, p=0.011). In addition, in the TT group of the *TRPV1* rs8065080, a moderate and negative correlation was observed between adding salt at the table and salt taste recognition threshold (r_s=-0.636, p=0.048).

4. Discussion

4.1. Genetics of the BP response to sodium loading, salt taste perception and salt intake

Findings from the present study suggest a genetic predisposition to salt sensitivity in the study population. Despite the small sample size, salt-sensitive increase in BP was detected. Moreover, other studies with similar sample sizes, 14-16 subjects respectively, have successfully investigated and detected this phenomenon in normotensive populations [42-44]. Finally, urinary markers of compliance with the diets, sodium and potassium, were satisfactory showing an overall good compliance with the diets.

SLC4A5 gene, coding for a sodium hydrogen bicarbonate transporter involved in sodium transport across the cellular membrane [45], affected salt-sensitive changes in BP. Carey et al. [19] noted that SNPs rs7571842 and rs10177833 had the most pronounced effects on salt sensitivity. One of these SNPs, rs7571842, had the greatest effect in this study population, increasing BP in individuals with AA genotype and confirming the protective effect of the G allele [19]. A *post hoc* power calculation revealed that, with the two-tailed 0.05 significance level, this test had a power of 92% to detect a difference in SBP between the two *SLC4A5* rs7571842 genotype groups (mean

values for ΔSBP 7.75 mmHg vs. 0.00 mmHg and standard deviations 2.87 mmHg vs. 1.06 mmHg). Regarding the rs10177833, the lack of confirmation of its effect may be due to its lower effect size that could potentially be detected in a larger sample size study. These results, however, align with Carey et al. [19] where the effect of rs10177833 on salt sensitivity observed in the University of Virginia (UVA) discovery cohort was not replicated in a HyperPATH study population. Other SNPs investigated in the present study were not associated with salt sensitivity in previous studies conducted in humans. The *SCNN1B* SNPs were associated with hypertension[24,25] but not salt sensitivity *per se* suggesting rs239345 may not have an effect on this specific phenotype in healthy population. Finally, the *TRPV1* rs8065080 appears to be functional and is associated with lower channel activity, a trait observed in salt-sensitive rats [20,23]. In this population, it did not have an effect on salt-sensitive changes in BP, suggesting that other variants in this gene may have more pronounced effects on BP.

Nevertheless, the A allele of the *SLC4A5* rs7571842 is present in approximately half of the European descent population with a third of the population having the risky AA genotype [38]. Additionally, salt-sensitive rise in BP, following a high sodium diet, was expressed as a continuous variable. The risk of CVD increases continuously and with each 2 mmHg increase in SBP there is a 7% increase in risk of mortality from IHD and a 10% increase in the risk of mortality from stroke [46]. The increase in SBP in healthy subjects with the rs7571842 AA genotype was 7.75 mmHg, which emphasises the clinical relevance of these results. Moreover, it has been estimated that approximately a third of deaths attributed to BP occur in individuals with BP lower than the hypertensive range [47]. They may represent a salt-sensitive part of the population which reflects salt sensitivity prevalence of 36% in this study. Considering the discrepancies in methods used in previous studies, it is difficult to draw any conclusion whether this prevalence could be expected in other populations with similar characteristics. Salt sensitivity prevalence of 26% in normotensives was established using an intravenous protocol for diagnosis of salt sensitivity [27]. However, more recent work suggests that this method can lead to misclassification and incorrect diagnosis [39,40]. Another potential issue in comparison of different study results is the BP measurement. While most studies still use the conventional measurements, from the studies that employ 24-hour BP measurements only a limited number is investigating salt sensitivity solely in healthy, normotensive populations [48-50].

It should be noted, however, that this study primarily investigated the effects of sodium loading on BP and as such, the above-described salt sensitivity prevalence should be regarded with

caution. When identifying subjects as salt-sensitive or salt-resistant it is recommended that the low and high sodium diets should be administered in a random order to achieve maximal reproducibility [35]. When a low sodium period precedes high sodium period RAAS may not be uniformly suppressed [51]. This may result in an increased BP response on a low sodium diet and would require larger sample size compared to the one in this study to detect the true effect of dietary sodium manipulation on BP and estimate the salt sensitivity prevalence. Therefore, if the order of the diets was randomised and high sodium diet preceded the low sodium diet in a proportion of the study population, the RAAS may have been supressed to an extent where more uniformity in the BP response to dietary intervention may have been observed. This in turn, may have resulted in a statistically significant difference in BP when transitioning from the low to the high sodium diet in the total study population.

Besides observed genetic predisposition to salt sensitivity of BP, the *SLC4A5* rs10177833 was associated with salt intake. With increasing number of A alleles there was a trend towards an increased energy adjusted sodium intake. The highest proportion of subjects in the second and third tertile of energy adjusted sodium intake was in the AA genotype group with the majority of these subjects (85%) having absolute sodium intake above the recommendations [5,52]. Recently, Smith et al. [53] have reported how individuals with enhanced bitter taste perception genotype (GC and GG alleles for the bitter taste receptor gene *TAS2R38*) were significantly more likely than CC homozygotes to have daily sodium intake higher than recommended. Furthermore, Kho et al. [54], in their genome wide association study (GWAS) have reported on several variants associated with salt intake. These variants were in genes coding for sodium, potassium and calcium channels, suggesting that genes coding for sodium transport proteins may be associated with increased salt intake, similar to the findings of this study. The mechanism behind this association is to be explored. It is not to exclude the potential expression of this cotransporter in taste receptor cells, as other sodium-dependent transporters primarily expressed in other tissues have been localised in tongue [55,56]. However, impaired sodium metabolism was reported as a consequence of rs10177833 induced increase in the *SLC4A5* transcription under conditions of high sodium intake [57]. Considering its strong linkage disequilibrium (LD) with rs7571842 [19], these two SNPs are most likely inherited together making the carriers of this genotype at increased risk of developing hypertension and CVD.

Moreover, there was no genetic predisposition to altered salt taste perception. The discrepancy in the results of the present study and the one by Dias et al. [8] may be explained by the

difference in thresholds measured. The taste quality of salt stimulus can be concentration dependent[58,59] which may explain the associations observed with suprathresholds in Dias et al. [8] but not with lower concentrations (STDT, STRT) used in this study. Nevertheless, the borderline non-significant trend observed for the *TRPV1* rs8065080 may be detected in a larger sample size study. For such study to be clinically meaningful, in addition to salt taste perception, dietary salt intake should be measured, as acknowledged by Dias et al.[8]. It has been shown that the reduction in salt intake results in important falls in BP, in both hypertensive and normotensive salt-sensitive individuals [2], and a reduction in overall CVD risk [1].

4.2. Associations between salt sensitivity, salt taste perception and salt intake

Together with the observed effect of genetics, salt sensitivity expressed as a change in BP after sodium loading was associated with taste thresholds for salt. In subjects that had complete dietary intervention data PP was positively associated with STDT. PP is the difference between SBP and DBP and is argued to be a better predictor of cardiovascular risk than SBP [60]. PP may be genetically determined by the *SLC4A5* rs7571842 [61]. The mechanisms behind this association and the causality remain unknown. However, the hypothesis was that genetics may play a role in this relationship which aligns with the finding that this association was observed only in certain genotype groups of the *SLC4A5* SNPs. This sub-group analysis should, nevertheless, be replicated in a study with a larger sample size in each genotype group, to achieve appropriate statistical power, and as such considered preliminary in this study.

Sakamoto et al. [26] reported that the ENaC activity may be the link between salt taste sensitivity and salt sensitivity of BP in animals. However, the *SCNN1B* rs239345 was not associated with salt sensitivity or salt taste thresholds in this study. In a larger sample size study potential effect of interactions between the *SLC4A5* and ENaC SNPs may be investigated and may provide insight into the mechanism behind this relationship. Nevertheless, the relevance of these findings lies in the actual relationship between salt taste thresholds and salt intake.

If there is a positive association between the thresholds for salt and salt-sensitive changes in BP, it can be theorised that salt-sensitive individuals with higher thresholds are at greater risk of developing hypertension due to their higher salt intake. In the present study, however, neither detection nor the recognition threshold for salt have been associated with total habitual dietary salt intake. Nevertheless, discretionary salt use accounts for approximately 15% of salt intake in

Western countries [62] and the results of the present study suggest it may be associated with salt taste thresholds. The association between salt taste perception and discretionary salt use may depend on the *SLC4A5* and *TRPV1* genotype, however these sub-group analyses should be replicated in a larger size study. This would, nonetheless, be in line with the notion that reduced salt taste sensitivity (i.e. higher salt taste threshold) drives individuals to consume more salt until reaching the salt concentration identified as pleasant [14]. Conversely, improved ability to taste salt when the taste of salt is deemed pleasant may result in increased salt intake. Indeed, research suggests that the preference for salty taste may be one of the factors affecting salt intake in younger populations and that discretionary salt use is more frequent in younger compared to older populations [63, 64]. Moreover, when salt content of processed food is reduced, consumers compensate its apparent lack by increasing the discretionary salt use [65]. Considering the evolving food supply and dietary habits of the UK population and worldwide, a better understanding of this behaviour could enable more targeted and effective public health interventions to reduce salt intake.

4.3. Strengths and limitations

This study has several strengths and limitations. A strength is the salt sensitivity phenotyping procedure with the dietary control of sodium intake. Moreover, a 24-hour ABPM procedure to determine the difference in BP between the diets provides many more measurements than conventional BP measurement reflecting usual BP more accurately. It also allows identification of individuals with a 'white coat' response or masked hypertension, and is a stronger predictor of cardiovascular morbidity and mortality than conventional measurement [34]. One of the limitations is a use of a FFQ to determine dietary salt intake. Even though FFQ represents dietary intake over a longer time-period, it relies heavily on respondents' honesty and long-term memory. However, sodium intake was energy adjusted, improving measurement accuracy. Freedman et al. [66] suggest that the attenuations and correlations with truth for the FFQs are improved when considering sodium densities, utilised in this study. Regarding the associations between genetics and variables of interest, where possible, a Cochran-Armitage test of trend was used. The advantage of the Cochran-Armitage trend test is that it is not dependent on the Hardy-Weinberg equilibrium assumption and is suggested as the genotype-based test for association [67-69]. Finally, the small sample size in sub-group analyses of the correlations between salt taste perception, BP response to sodium loading and salt intake warrants replication of these results in a larger sample size study.

In conclusion, this preliminary data suggests there is a genetic predisposition to salt sensitivity in healthy, adult Caucasians. The *SLC4A5* rs7571842 was confirmed as the variant with the effect on salt-sensitive changes in BP. Another *SLC4A5* variant, rs10177833, most likely inherited together with the rs7571842, is associated with salt intake. Moreover, the observed associations between salt taste perception and salt sensitivity, together with the association between salt taste perception and discretionary salt use may depend on the *SLC4A5* and *TRPV1* genotype. Since there was no association between genetics and salt taste perception, the mechanisms behind these associations are to be further explored together with gene-gene interactions. Nevertheless, preference for salty taste may be a driver of salt intake in younger populations and warrants further investigation. Studies investigating these associations should comprehensively explore all potential variables, such as genetic predisposition, salt taste perception and salt intake to contribute towards more successful prevention of hypertension and CVD.

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Conflict of interest

None.

Authorship

Y.M and L.P. designed the experiment. L.P. conducted data collection, data analysis and wrote the paper. Y.M supervised the project. Both authors discussed the results and implications and commented on the manuscript at all stages.

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Tables

Table 1. Baseline characteristics of study subjects, total sample (n=20) and according to salt sensitivity status (n=14). Data presented as mean and SEM or absolute (relative) frequencies. P value for difference between salt-sensitive and salt-resistant subjects (Independent samples t-test, Fischer's exact test).

	Total (n=20)		**Salt-sensitive (n=5)**		**Salt-resistant (n=9)**		**p**
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	
Age (years)	28.0	(10.5)[a)]	35.8	4.6	33.2	2.7	0.612
Sex							
Male	8 (40)		2 (40)		2 (22)		0.580
Female	12 (60)		3 (60)		7 (78)		
Race							
White	16 (80)		4 (80)		6 (67)		0.999
Other	4 (20)		1 (20)		3 (33)		
BMI (kg/m^2)	23.9	0.7	24.7	1.9	23.7	0.7	0.633
SBP (mmHg)	121.3	3.0	125.8	9.2	118.2	4.4	0.413
DBP (mmHg)	70.4	2.1	71.9	6.3	71.2	2.9	0.913
Smoking status							
Yes	1 (5)		1 (20)		0		0.357
No	19 (95)		4 (80)		9 (100)		
Physical activity level							
Active	15 (75)		2 (40)		7 (78)		0.413
Moderately active	1 (5)		1 (20)		0		
Moderately inactive	2 (10)		1 (20)		1 (11)		
Inactive	2 (10)		1 (20)		1 (11)		

DBP, diastolic blood pressure; SBP, systolic blood pressure

a), median (interquartile range)

Table 2. Clinical characteristics of study subjects (n=14) on low- and high-salt diet (mean and SEM). P values for difference between low- and high-salt diets (Paired samples t-test).

	Low-salt diet		High-salt diet		p
	Mean	**SEM**	**Mean**	**SEM**	
SBP (mmHg)	113.6	2.7	115.8	3.0	0.107
DBP (mmHg)	66.9	1.4	68.6	2.2	0.261
MAP (mmHg)	82.5	1.6	84.4	2.4	0.170
PP (mmHg)	46.7	2.2	47.2	1.8	0.656
Urine sodium excretion (mmol/24 hour)	66.1	8.9	281.5	24.4	3.3×10^{-7}
Urine potassium excretion (mmol/24 hour)	75.8	5.5	81.8	5.8	0.243

DBP, diastolic blood pressure; MAP, mean arterial pressure, PP, pulse pressure; SBP, systolic blood pressure

Table 3. Correlation analysis between salt taste thresholds (mol/l) and mean change in BP (mmHg) from low- to high-salt diet, and salt taste thresholds (mol/l) and dietary sodium intake (mg sodium per 1000 kcal) (n=14)

	ΔSBP	**ΔDBP**	**ΔMAP**	**ΔPP**	**Sodium intake**
STDT	0.098 (0.740)	-0.377 (0.185)	-0.303 (0.293)	0.551 (0.041)	-0.016 (0.956)
STRT	0.403 (0.153)	0.209 (0.473)	0.260 (0.370)	0.039 (0.895)	-0.113 (0.700)

DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; STDT, salt taste detection threshold; STRT, salt taste recognition threshold; SBP, systolic blood pressure

Spearman rho (p value)

689 **Figure legends**

690

691 Figure 1. Overview of the study procedure.

692 Footnotes: ABPM, ambulatory blood pressure monitoring device; BP, blood pressure

693

694 Figure 2. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure
695 (MAP) change from low- to high-salt diet according to *SLC4A5* rs7571842 (a) and rs10177833 (b)
696 genotype status (n=14). Analysis conducted on the following model: major allele homozygote
697 versus heterozygote plus minor allele homozygote. Error bars represent + SEM. (Independent
698 samples t-test, *Mann-Whitney U test).

699 Footnotes: DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood
700 pressure

701

702 Figure 3. Proportion of subjects (n=20) with low and high salt taste recognition thresholds
703 according to *SLC4A5* rs7571842 (a) and rs10177833 (b), *SCNN1B* rs239345 (c) and *TRPV1*
704 rs8065080 (d) genotype. Open bars represent low threshold and closed bars high threshold (Cochran
705 Armitage test of trend).

706

707 Figure 4. Proportion of subjects (n=20) in the different tertiles of energy adjusted sodium intake
708 according to *SLC4A5* rs7571842 (a) and rs10177833 (b), *SCNN1B* rs239345 (c) and *TRPV1*
709 rs8065080 (d) genotype. Open bars represent first tertile (< 1241 mg/1000 kcal) and closed bars
710 second + third tertile combined (≥ 1241 mg/1000 kcal) (Cochran Armitage test of trend).

711

712 Figure 5. Correlation between salt taste thresholds and discretionary salt use according to *SLC4A5*
713 rs7571842 (n=6) and *TRPV1* rs8065080 (n=10) genotypes. Adding salt while cooking/table; 1-
714 always, 2-usually, 3-sometimes, 4-rarely, 5-never (Spearman's correlation).

715