Genetic and physiological markers of salt sensitivity and its effects on salt taste perception and intake
Outline

Background
- Hypertension and salt sensitivity (SS)
- Why salt taste perception and intake?

Part 1. SS, salt taste perception, salt intake
- Genetic predisposition to SS
- Genetic predisposition to impaired salt taste perception and increased salt intake

Part 2. Protein expression in SS
- SNP – protein expression
- Urinary exosome protein isolation and ELISA

Future prospects
- Future research on salt taste perception and intake
- SS biomarker?
Hypertension

• Hypertension prevalence in Europe and globally 30-40%
• Hypertension responsible for at least 45% of deaths due to heart disease and 51% of deaths due to stroke
• Major contributor: excessive salt intake
• Salt intake in the EU ranging between 7 g and 18 g/day – exceeding recommended intake

WHO Regional office for Europe, 2013
Salt sensitivity

- Salt-induced increase in blood pressure
- 51% of hypertensive and 26% of people with normal blood pressure are salt-sensitive
- Independent CVD and mortality risk factor!


N – normal blood pressure
H – high blood pressure
R – salt-resistant
S – salt-sensitive
Interplay of mechanisms

Pilic et al. (2016) Nutr Rev 74(10), 645-658

ANP, atrial natriuretic peptide; GFR, glomerular filtration rate; NKCC2, sodium-potassium-chloride cotransporter; NO, nitric oxide; RAAS, renin-angiotensin aldosterone system; SNS, sympathetic nervous system
Why salt taste perception/sensitivity and intake?

• Salt sensitivity – clear example of a gene-diet interaction
• Taste one of the main determinants of food intake
• Salt sensitivity suggested as the mediating variable in salt taste sensitivity – BP relationship
• Salt sensitivity, salt taste sensitivity (perception) and salt intake not studied comprehensively
Part 1.
Genetics of salt sensitivity and salt taste perception – the choice of SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC4A5 (electrogenic sodium</td>
<td>rs7571842</td>
<td>Blood pressure, pulse pressure, salt sensitivity (humans); may act jointly with ENaC (animals)</td>
<td>Carey et al., 2012 Hunt et al., 2006</td>
</tr>
<tr>
<td>bicarbonate cotransporter)</td>
<td>rs10177833</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCNN1B (β-subunit of the ENaC)</td>
<td>rs239345</td>
<td>Salt taste suprathreshold sensitivity (humans), hypertension (humans)</td>
<td>Dias et al., 2013 Hannila-Handelberg et al, 2005</td>
</tr>
<tr>
<td>TRPV1 (ion channel/capsaicin</td>
<td>rs8065080</td>
<td>Salt taste suprathreshold sensitivity (humans), TRPV1 downregulation - salt sensitivity (animals)</td>
<td>Dias et al., 2013 Hao et al., 2011 Wang et al., 2006</td>
</tr>
<tr>
<td>receptor)</td>
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</table>
Salt sensitivity status – AHA recommendations

• Twenty healthy young to middle-aged subjects
• Low-salt diet for 7 days (3 g of salt/day)
• High-salt diet for additional 7 days (18 g salt/day)

• 24-hour automated blood pressure monitoring
• 24-hour urine sample for sodium, potassium and creatinine excretion
Salt taste perception and intake

BS ISO3972:2011

**STDT** - the lowest concentration of the sample where the subject can consistently perceive an impression but not identify the taste.

**STRT** - the sample concentration where the subject consistently perceives the taste as salty.

Salt intake (mg of sodium per 1000 kcal)
Genetic predisposition to salt sensitivity

**rs7571842**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mean change in blood pressure (mmHg)</th>
<th>*SBP, p=0.002; ΔDBP, p=0.044; ΔMAP, p=0.014</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (n=4)</td>
<td><img src="a" alt="Graph" /></td>
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</tr>
<tr>
<td>AG + GG (n=10)</td>
<td><img src="a" alt="Graph" /></td>
<td></td>
</tr>
</tbody>
</table>

**rs10177833**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mean change in blood pressure (mmHg)</th>
<th>ΔSBP, p=0.198; ΔDBP, p=0.221; ΔMAP, p=0.202</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (n=6)</td>
<td><img src="b" alt="Graph" /></td>
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</tr>
<tr>
<td>AC + CC (n=8)</td>
<td><img src="b" alt="Graph" /></td>
<td></td>
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</table>
Genetics, salt taste perception and intake

TRPV1 rs8065080 – salt taste perception

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>% study subjects</th>
</tr>
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<tbody>
<tr>
<td>TT</td>
<td>80</td>
</tr>
<tr>
<td>CT</td>
<td>50</td>
</tr>
<tr>
<td>CC</td>
<td>30</td>
</tr>
</tbody>
</table>

p = 0.078

SLC4A5 rs10177833 – salt intake

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>% study subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (n=8)</td>
<td>60</td>
</tr>
<tr>
<td>AC (n=8)</td>
<td>80</td>
</tr>
<tr>
<td>CC (n=4)</td>
<td>50</td>
</tr>
</tbody>
</table>

p = 0.037

Proportion of subjects (n=20) with low and high salt taste recognition thresholds

Proportion of subjects (n=20) in different tertiles of sodium intake.
Associations between salt sensitivity, salt taste perception and salt intake

- SLC4A5 - salt-sensitive increase in BP, increased salt intake
- No association between genetics and salt taste perception
- Association between salt taste perception and intake depends on the genotype.
- Negative correlation between salt taste recognition threshold and the frequency of adding salt at the table in the rs8065080 TT group - preference for salty taste?
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Part 2. Protein expression in salt sensitivity

- Sodium bicarbonate cotransporter (SLC4A5/NBCe2) as the protein of interest
- rs7571842 in strong LD with rs7583544 – transcriptional regulation? (F-SNP)

- Issues with salt sensitivity diagnosis
- Inability to conduct GWAS
- Genotype information not sufficient
Urinary exosome protein expression

- NHE3, SLC4A4 and other members of the SLC family in the urinary exosome protein database

- SLC4A5?

- The aim: To isolate and measure the SLC4A5 protein expression from urinary exosomes and determine if there is a difference in expression according to rs7571842 genotype and salt sensitivity status
To date...

- Urine samples collected from 4 subjects diagnosed for salt sensitivity (rs7571842 AA and GG)
- Exosomes isolated using a nanomembrane method by Cheruvanky et al. (2007)
- The ELISA issues
- But...
Future research

• Explore gene-gene interactions in salt sensitivity, salt taste sensitivity and salt intake (also causality and mechanism)
• A follow-up study utilising a different method of measuring salt intake
• Optimise the exosome isolation method for ELISA measurement – potential for salt sensitivity biomarker?
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