Genetic determinants of blood pressure responses to caffeine drinking

Running head: nutrigenetics of blood pressure responses to caffeine

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ONLINE SUPPLEMENTAL MATERIAL

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LEGEND TO ONLINE SUPPLEMENTAL MATERIAL FIGURES

Online Supplement Figure 1. Forest plot of the effect of genetic variants on the risk of having an above-the-median difference ($\Delta$) in *peak systolic blood pressure* (SBP) between caf and decaf. Genetic variants investigated in the study are listed on the left-hand side, grouped according to the gene where they are located. The plot shows, for the homozygotes of each single genetic variant, the specific effect on BP as odds ratios (ORs) and 95% confidence intervals (CI, horizontal lines) against both the alternative genotypes at the same locus. The homozygotes not shown (AMPD1 TT, ADRB2 164IleIle e ADRB3 64ArgArg) are not present in our population. Data were analyzed by a conditional logistic regression model using BP variables as discrete; the null hypothesis of no association was tested with the likelihood ratio test (LRT). The vertical line represents no effect. The overlap of the CI with this line indicates that the effect size does not deviate significantly from no effect. “Caf” stands for decaffeinated coffee preparation plus 3 mg/kg caffeine; “decaf” stand for decaffeinated coffee. N= 110 subjects both after caf than after decaf intake.

Online Supplement Figure 2. Forest plot of the effect of genetic variants on the risk of having an above-the-median difference ($\Delta$) in *mean diastolic blood pressure* (DBP) between caf and decaf. Genetic variants investigated in the study are listed on the left-hand side, grouped according to the gene where they are located. The plot shows, for the homozygotes of each single genetic variant, the specific effect on BP as odds ratios (ORs) and 95% confidence intervals (CI, horizontal lines) against both the alternative genotypes at the same locus. The homozygotes not shown (AMPD1 TT, ADRB2 164IleIle e ADRB3 64ArgArg) are not present in our population. Data were analyzed by a conditional logistic regression model
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Online Supplement Figure 3. Forest plot of the effect of genetic variants on the risk of
having an above-the-median difference (Δ) in peak diastolic blood pressure (DBP) between
caf and decaf. Genetic variants investigated in the study are listed on the left-hand side,
grouped according to the gene where they are located. The plot shows, for the homozygotes of
each single genetic variant, the specific effect on BP as odds ratios (ORs) and 95% confidence
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“stands for decaffeinated coffee preparation plus 3 mg/kg caffeine; “decaf” stand for
decaffeinated coffee. N= 110 subjects both after caf than after decaf intake.
Table 1. Gene variants (polymorphism) analyzed, their known biological effects, corresponding proteins and effectors, primers and annealing temperature used for PCR.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Effector</th>
<th>Genetic variant</th>
<th>rs Number</th>
<th>Biological effect of genetic variant</th>
<th>Primer</th>
<th>Annealing temperature</th>
</tr>
</thead>
</table>
| CYPIA2  | hepatic metabolism | caffeine demethylation | 734 A>C | rs762551 | decreases enzyme inducibility \(\rightarrow\) impaired caffeine metabolism (9) | Forward: CCAAGAAGGAGCAAGTCA
Reverse: CAGTTCAGAAGATGGATC | 63°C |
| ADORA2A | mental arousal, psychomotor effect mediated by dopaminergic neurons, vasodilation, platelet aggregation | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | 1096 C>T | rs5751876 | susceptibility to anxiety (12) and sleep changes (7) | Forward: CCAAGGCCAAGAATGCC
Reverse: CCAAAAGGAGCATCAGA | 60°C |
| AMPD1  | energy metabolism, vasodilation | AMP hydrolyase activity (deamination of AMP to IMP) | C34T | rs17902729 | reduced activity of the enzyme (13) \(\rightarrow\) increase in adenosine availability for its receptors (14) | Forward: GCTATCTACATCTATCTATCC
Reverse: TACATGGTCTTCTGAATTA | 61°C |
| ADRA1A | cardiac positive inotropism, vasoregulation | G-protein \(\rightarrow\) ↑ IP3 and DAG \(\rightarrow\) ↑ intracellular Ca | Arg147Cys | rs1048101 | no information on cardiovascular effects (17) | Forward: ATGCTCCAGCCAAGAGTTCA
Reverse: TCCAAGAAGAGCTGGCCTTC | 60°C |
| ADRA2B | inhibition of neurotransmitters delivery | G-protein \(\rightarrow\) adenylate cyclase inhibition \(\rightarrow\) ↓ cAMP | In+910Del | rs29009568 | reduced desensitization \(\rightarrow\) increased vasoconstriction induced by its activation (6) | Forward: AGGCTGTTTGGGGCAATTCT
Reverse: CAAACTGAGGCAGACACT | 60°C |
| ADRB1  | cardiac positive inotropism and chronotropism \(\rightarrow\) regulation of cardiac output and heart rate | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Arg389Gly | rs1801253 | greater activity of adenylate cyclase in vitro after stimulation with isoproterenol (14,26) | Forward: GTCGCCGCCCGCCTCGTT
Reverse: CCATGCCCGCTGTCCACTGCT | 58°C |
| ADRB1  | cardiac positive inotropism and chronotropism \(\rightarrow\) regulation of cardiac output and heart rate | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Ser49Gly | rs1801252 | no functional effects identified (21) | Forward: GTGGCGCGCCGCCGTCTC
Reverse: CCAGATTGACGCAGGACT | 58°C |
| ADRB2  | smooth muscle relaxation \(\rightarrow\) vasodilation | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Arg164Ile | rs1042713 | increased downregulation by the agonist (22), effect on vascular reactivity (25) | Forward: GGAACGCGACGGCTCTCTC
Reverse: CCAGATTGACGCAGGACT | 58°C |
| ADRB2  | smooth muscle relaxation \(\rightarrow\) vasodilation | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Glu27Gln | rs4994 | agonist-induced downregulation, effect on vascular reactivity (25) | Forward: GGACTTTTGGCAACTTCTGG
Reverse: ACGAAGACGACGAGACAGAT | 58°C |
| ADRB3  | smooth muscle relaxation \(\rightarrow\) vasodilation | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Thr164Ile | rs1042714 | decreased ligand affinity and intensity of stimulation (19,20) | Forward: GTGGCGCGCCGCCGTCTC
Reverse: CCAGATTGACGCAGGACT | 58°C |
| ADRB3  | lipolysis activation | G-protein \(\rightarrow\) adenylate cyclase activation \(\rightarrow\) ↑ cAMP | Thr94Arg | rs4994 | association with essential hypertension (23) | Forward: GGAACGCGACGGCTCTCTC
Reverse: CCAGATTGACGCAGGACT | 58°C |

rs = ref single-nucleotide polymorphism. CYPIA2 = cytochrome P450 1A2. ADORA2A = adenosine receptor 2A. AMPD = adenosine monophosphate deaminase. ADRA1A = \(\alpha\)1A adrenergic receptor. ADRA2B = \(\alpha\)2B adrenergic receptor. ADRB1 = \(\beta\)1 adrenergic receptor. ADRB2 = \(\beta\)2 adrenergic receptor. ADRB3 = \(\beta\)3 adrenergic receptor. IP3 = inositol trisphosphate. cAMP = cyclic adenosine monophosphate.
Odds Ratio for \( \Delta SBP \) Peak above Median

- CYP1A2*1F
- CYP1A2*1A
- ADORA2A - CC
- ADORA2A - TT
- AMPD1 - CC
- ADRA1A - 347ArgArg
- ADRA1A - 347CysCys
- ADRA2B - II
- ADRA2B - DD
- ADRB1 - 49SerSer
- ADRB1 - 49GlyGly
- ADRB1 - 389ArgArg
- ADRB1 - 389GlyGly
- ADRB2 - 16GlyGly
- ADRB2 - 16ArgArg
- ADRB2 - 27GluGlu
- ADRB2 - 27GlnGln
- ADRB2 - 164ThrThr
- ADRB3 - 64TrpTrp

Online Supplement Fig. 1
Odds Ratio for \[\Delta\text{DBP Mean above Median}\]

- CYP1A2*1F
- CYP1A2*1A
- ADORA2A - CC
- ADORA2A - TT
- AMPD1 - CC
- ADRA1A - 347ArgArg
- ADRA1A - 347CysCys
- ADRA2B - II
- ADRA2B - DD
- ADRB1 - 49SerSer
- ADRB1 - 49GlyGly
- ADRB1 - 389ArgArg
- ADRB1 - 389GlyGly
- ADRB2 - 16GlyGly
- ADRB2 - 16ArgArg
- ADRB2 - 27GluGlu
- ADRB2 - 27GlnGln
- ADRB2 - 164ThrThr
- ADRB3 - 64TrpTrp

Odds Ratio for \[\Delta\text{DBP Mean above Median}\]

Online Supplement Fig. 2
CYP1A2*1F
CYP1A2*1A
ADOR A2A - CC
ADOR A2A - TT
AMPD1 - CC
ADOR A1A - 347ArgArg
ADOR A1A - 347CysCys
ADOR A2B - II
ADOR A2B - DD
ADOR B1 - 49SerSer
ADOR B1 - 49GlyGly
ADOR B1 - 389ArgArg
ADOR B1 - 389GlyGly
ADOR B2 - 16GlyGly
ADOR B2 - 16ArgArg
ADOR B2 - 27GluGlu
ADOR B2 - 27GlnGln
ADOR B2 - 164ThrThr
ADOR B3 - 64TrpTrp

Odds Ratio for ∆DBP Peak above Median

Online Supplement Fig. 3