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Hydrochlorothiazide efficacy and polymorphisms in ACE, ADD1 and GNB3 in healthy, male volunteers

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Abstract *Objective:* The antihypertensive effect of thiazide diuretics has recently been associated with genetic variation in the angiotensin I-converting enzyme (ACE), alpha-adducin (ADD1) and the G protein subunit $\beta 3$ (GNB3). Analysis of short-term diuretic effects may provide insight into the mechanisms behind these findings. *Methods:* A total of 103 male volunteers took 25 and 100 mg hydrochlorothiazide (HCT) after a placebo day, each. We measured volume, sodium, chloride, potassium, calcium excretion, blood pressure and heart rate. *Results:* Excretion and cardiovascular parameters were highly constant between the 2 placebo days. The resting heart rate was 2–3 beats/minute (bpm) higher per ACE insertion allele on all 4 study days. The HCT-induced excretion of sodium, chloride and volume was independent of the genotypes. The additional potassium excretion induced by 100 mg HCT was 44 ± 21 , 33 ± 27 and 16 ± 26 mmol (mean \pm SD, $p < 0.001$) in ACE II, ID and DD carriers and the same trend was observed after 25 mg HCT. As a second finding, the 100 mg HCT-induced calcium retention was 0.2 ± 1.2 , 0.7 ± 0.8 and 1.7 ± 2.1 mmol in ADD1 Gly/Gly, Gly/Trp and Trp/Trp carriers ($p = 0.002$) and the same trend existed after 25 mg HCT. *Conclusion:* The effects of genetic polymorphisms were stronger with the higher diuretic dose. ACE insertion allele carriers appeared to be more prone to hypokalaemia than deletion allele carriers. ADD1 Trp460 carriers may especially benefit from the calcium-sparing effect of thiazides. Both associations should be further studied in long-term treatment with thiazide diuretics.

Keywords Genetic polymorphisms · ACE I/D · ADD1 · GNB3 · Hydrochlorothiazide · Diuretics

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Introduction

Recent study results highlighted the importance of thiazide diuretics as antihypertensive drugs [1]. Despite variation in the response to thiazide diuretics as a well-known therapeutic problem, only a few studies on the pharmacogenetic determinants of individual diuretic efficacy have been performed [2–4]. Thiazide diuretics block the renal sodium chloride channel (NCC) and thereby increase sodium and chloride excretion, which is thought to be the major determinant of the antihypertensive effect [5]. Net increases in volume and potassium excretion are considered to be secondary consequences of the increased NaCl excretion. In contrast to loop and K^+ -sparing diuretics, thiazides decrease the excretion of calcium making them useful in osteoporosis therapy [6–8]. A prototypic thiazide diuretic is hydrochlorothiazide (HCT). HCT is well absorbed, little bound to plasma proteins and mainly excreted unchanged in the urine. The plasma half-life of HCT is about 10 h with a renal clearance of the unbound fraction of about 850 ml/min [2, 9, 10].

To investigate potential effects on the short-term diuretic drug response, we selected those three polymorphisms that are known to be functional or strongly associated with a functional polymorphism and that were reported to be associated with the blood pressure response to a diuretic in at least one study each. One is the angiotensin I-converting enzyme (ACE) insertion/deletion polymorphism (ACE I/D). ACE I/D, although not considered functional, has been associated with plasma and tissue ACE levels [11]. The ACE serum levels were 299 ± 49 , 392 ± 67 and 494 ± 88 $\mu\text{g/l}$ in II, ID and DD subjects and thus there was an almost linear gene-dose relationship [12]. The long-term outcome of antihypertensive therapy was not associated with the ACE I/D in the GenHAT study [13]. On the other hand, a recent 2-month study reported that subjects carrying the ACE insertion allele responded better to HCT than carriers of the deletion allele [14]. We hypothesised that the ACE I allele may predispose to a stronger diuretic effect.

The alpha-adducin (ADD1) Trp460 allele has been associated with faster transmembranous sodium transport in a

gene-dose dependent manner compared to the Gly460 allele when measured *ex vivo* in erythrocytes [15]. The Trp allele has also been associated with a salt-sensitive form of hypertension [16]. Blood pressure response to 1 or 2 months of HCT treatment was stronger in carriers of the tryptophan allele in three studies [14–16] in contrast to negative findings in another study [17]. We hypothesised that the tryptophan allele also predisposes to a stronger diuretic effect.

The G protein β subunit 3 (GNB3) C825T polymorphism was frequently studied as an index single nucleotide polymorphism of a specific haplotype that has been associated with truncated β subunit splice variants with increased biological activity. A number of diseases appeared to be associated with this polymorphism [18]. In one study, the blood pressure reduction induced by 4 weeks of HCT treatment was greater in T allele carriers [19]. From these prior data we hypothesised that the GNB3 T allele may predispose to a stronger diuretic effect.

We aimed to approach a better understanding of the potential effects of these three polymorphisms on the thiazide-induced blood pressure reduction. We performed a single-blind, placebo-controlled study with two single HCT doses, 25 and 100 mg, to measure volume and ion excretion and short-term effects on cardiovascular parameters.

Methods

Study conduct

The study protocol was approved by the Ethics Committee of the University of Göttingen. All subjects gave written informed consent after oral explanation of the study procedures. The study was performed in 103 healthy, male volunteers. Confinement to the male gender group was due to practical reasons and as most former studies with HCT focussed on males. The two HCT doses were administered in a randomized order separated by at least 7 days. Each occasion consisted of a 24-h diet run-in phase, placebo on the 2nd and verum on the 3rd day. The subjects were unaware on which day they took placebo or verum but the sequence (diet run-in, placebo, verum) was always the same. The order of 25 or 100 mg HCT was randomised and the subjects were unaware of which dose they took.

Placebo and verum days were conducted identically. Urine was collected from 0–1, 1–3, 3–6, 6–10 and 10–24 h after HCT intake. The subjects remained on the ward for the 1st h and thereafter returned to the ward at the end of each collection interval, where they completed the urine collection for the respective interval. Urine sampling was considered complete when the 24-h creatinine excretion on the study day was within 80–120% of the creatinine excretion on the placebo day and the interval-wise calculated creatinine clearance did not fall below 100 ml/min per 1.73 m² body surface. For blood pressure measurement 3 h after HCT intake a shortened version of the Schellong test was applied. The volunteers rested in a supine position for at least 5 min. For the next 5 min, blood pressure mea-

surements were performed in 1-min intervals with the subjects remaining in a supine position. The subjects then got up and repeated blood pressure measurements were performed while the volunteers were in an upright standing position. Finally, the subjects lay down again for at least 5 min. During the entire procedure heart rate and blood pressure were measured every 1 min.

Molecular genetic analyses

Genomic DNA was prepared from venous blood samples by automatic solid phase DNA extraction (EZ1 Bio-Robot, QIAGEN, Hilden, Germany). The DNA was adjusted to 10 ng/ μ l for the TaqMan-based assays. Genotyping of the ADD1 Gly460Trp (rs4961) was performed using the commercial TaqMan SNP genotyping assay C_11764545_20 (Applied Biosystems, Darmstadt, Germany). The genotyping of GNB3 825C>T was performed using customer SNP assays (Applied Biosystems) containing primers 5' AC CAGGAGCTGATCTGCTTCTC 3' and 5' TCGTCGTA GCCAGCGAATAGTAG 3' and probes 5' VIC-CACG TCCGTGGCC-MGB 3' and 5' 6-FAM-ACGTCTGTG GCCT-MGB 3'. Both TaqMan-based genotyping reactions were carried out in 5 μ l volume on 384-well plates using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) and standard conditions for a quantitative real-time polymerase chain reaction (PCR): preincubation 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The endpoint readings were done within 30 min and the results analysed using the allelic discrimination modus of the SDS 2.0 Software (Applied Biosystems). Controls in each run included DNA free reaction mixture and control DNAs carrying each of the tested genotypes. Genotyping of the ACE I/D 287 bp intronic insertion/deletion polymorphism was carried out modified according to [20, 21]. In brief, 1 μ l of undiluted genomic DNA was added to 24 μ l reaction mix containing 1 \times PCR buffer II (Applied Biosystems), 1 mM MgCl₂, 140 μ M dNTPs, 0.2 μ M of each ACEdel_Figowr (5' CT GCTGGAGACCACTCCCATCCTTTCT 3') and ACEdel_rev primer (5' GATGTGGCCATCACATTCGTCAG AT 3') and 1 U of the AmpliTaq DNA Polymerase (Applied Biosystems). The PCR reaction was performed in a Mastercycler Thermal Cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 2 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C and a terminal extension for 7 min at 72°C. The PCR products were directly analysed on 3% agarose gel. The deletion allele resulted in a signal at 196 bp while the non-deleted allele in a signal at 483 bp. To exclude a possible miscall of the heterozygote samples as homozygous deletion ones all the homozygous deletion samples were validated in an additional PCR reaction where ACEdel_rev primer was substituted with the insertion-specific ACEins_rev primer (5' TCGAGACCATCC CGGCTAAAAC 3'). In that case, only heterozygous but not homozygous deletion samples showed a PCR signal at 281 bp. Reanalysis of at least 20% of the samples showed

Table 1 Mean 24-h excretion (SD) and vital parameters 3 h after drug intake on the 4 study days

	Placebo day		HCT	
	1	2	25 mg	100 mg
Volume (ml)	3025 (1172)	2983 (1091)	3438** (1128)	3669** (1027)
Na ⁺ (mmol)	133 (62)	125 (65)	204** (83)	276** (96)
Cl ⁻ (mmol)	138 (58)	128 (58)	228** (81)	315** (96)
K ⁺ (mmol)	75 (28)	73 (26)	97** (31)	107** (32)
Ca ²⁺ (mmol)	4.8 (2.2)	4.8 (2.3)	4.3* (2.1)	4.5* (2.1)
Resting heart rate	66.4 (9.9)	66.5 (9.9)	66.8 (10.0)	69.4* (11.1)
Increase in heart rate	16.2 (9.2)	17.7 (8.1)	18.9 (11.2)	19.2 (9.0)
Resting mean arterial pressure ^a	76.0 (7.9)	76.0 (7.8)	75.2 (7.5)	76.0 (6.9)
Increase in mean arterial pressure ^a	6.4 (5.8)	5.6 (6.7)	6.0 (6.6)	3.7* (7.0)

* ** $p < 0.01$ and $p < 0.001$ compared to the corresponding placebo day (t -test)

^aMean arterial pressure calculated as $(RR_{\text{systolic}} + 2 \times RR_{\text{diastolic}}) / 3$

full concordance between the double determinations. All three genetic markers were in Hardy–Weinberg equilibrium.

Statistical methods

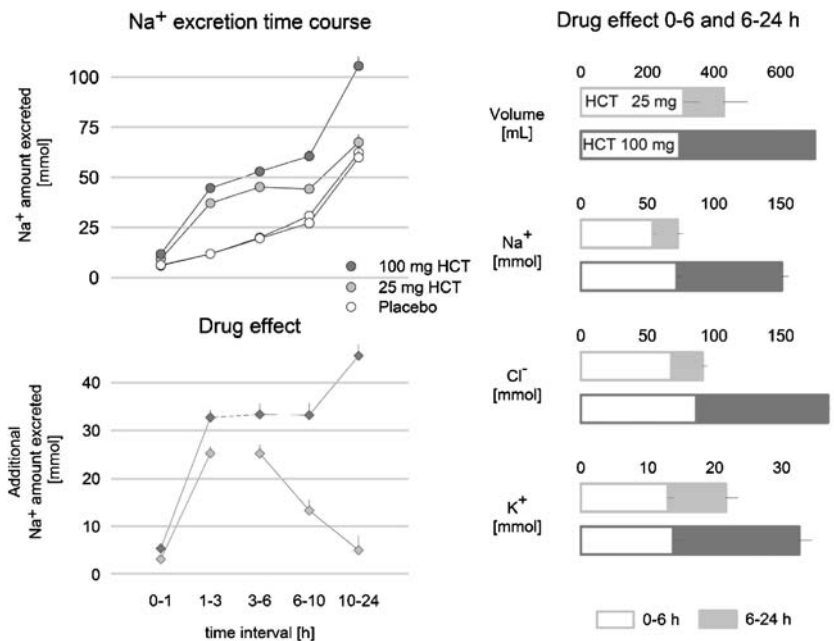
Standard statistical methods were employed using SPSS version 11.0. Repeated measures analysis of variance (ANOVA) was used to compare the 2 placebo days. The impact of the polymorphisms was analysed using two-sided single linear regression analysis, ANOVA and multiple linear regression analysis. A p value < 0.05 was considered significant. With 103 subjects with a power of 80% and $\alpha = 0.05$ a determination coefficient of 7.45% can be detected. Bonferroni α -adjustment for multiple testing of 5 parameters with 3 polymorphisms was used for the 15 main linear regression test.

Results

The study was performed in 103 healthy men aged between 18 and 49 years (mean \pm SD: 28 ± 7.5). The volunteers were between 167 and 201 cm tall (183 ± 7) and weighed between 58 and 100 kg (82 ± 10); their body mass index (BMI) ranged from 18.7 to 29.6 kg/m^2 (24.4 ± 2.4).

High intraindividual stability in volume and ion excretion between the 2 placebo days was observed (Table 1, Fig. 2). The time course over the five collection intervals was nearly indistinguishable between the 2 placebo days, exemplified for sodium excretion in Fig. 1. Repeated measures ANOVA attributed 60–90% of the overall variation of the excretion and cardiovascular parameters to interindividual variation, the variation source that includes the genetically determined variation. Less than 2% of overall variation were attributed to a systematic difference between the placebo days (Fig. 2).

Fig. 1 Volume and ion excretion over 24 h showing the mean (SEM) excreted ion amount fraction-wise. *Left side:* the mean (SEM) Na⁺ excretion for the five collection intervals. *Upper panel:* raw data on the 4 study days; *lower panel:* intraindividual differences between the 25 mg and 100 mg HCT days and their respective placebo days. *Right side:* the mean (SEM) of the intraindividual differences in excreted ion amounts between the 25 mg (light grey bars) and the 100 mg HCT (dark grey bars) days and their respective placebo days for the time intervals 0–6 h (open stack) and 6–24 h (filled stack)



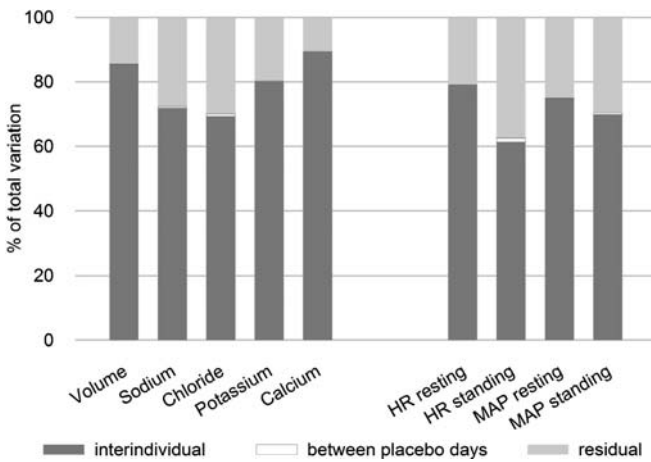


Fig. 2 Variation components in 24-h excretion and cardiovascular parameters on the 2 placebo days depicting the relative contribution of interindividual variation, intraindividual variation and residual variation to the overall variation derived by repeated measures ANOVAs of the 2 placebo days on the 24-h excretion of the six ions and of the heart rate (HR) and the mean arterial pressure (MAP) after 5 min lying in bed (resting) and the increase over consecutive 5 min standing (standing)

The doses of 25 and 100 mg HCT differentially induced the volume and ion excretion. The cumulative 24-h excretion increased in a dose-dependent manner (Table 1). The time course of volume, Na^+ , Cl^- and K^+ excretion was different between the 25 and the 100 mg dose study days (Fig. 1). The major part of the additional amount excreted that was induced by 25 mg HCT was excreted in the 0–6 h interval. In the 0–6 h interval the 100 mg HCT-induced excretion did not much exceed the 25 mg-induced excretion. In contrast the majority of the additional amount excreted induced by 100 mg HCT was excreted in the 6–24 h interval (Fig. 1). Calcium excretion was nearly indistinguishable between the 25 and the 100 mg dose in cumulative amount and time course. While between 0 and 6 h minor mean additional calcium excretion was induced, calcium retention was observed in the 6–24 h interval, which on average exceeded the additional calciuresis in the first interval. The distribution patterns of the saluretic effects induced by 25 and 100 mg HCT in no case deviated significantly from a normal distribution and no clear outlier groups could be identified.

There appeared to be an association of the 100 mg HCT-induced volume excretion with the ACE I/D polymorphism (Table 2) but no gene-dose-related trend was observed and after adjustment for multiple testing the relation was not significant. Also the additional sodium and chloride excretion was not significantly associated with the ACE I/D polymorphism. The 24-h additional K^+ excretion induced by 100 mg HCT was significantly associated with the ACE I/D polymorphism (linear regression $r^2=11.6\%$, $p=0.00056$, ANOVA $p=0.002$, Table 2, Fig. 3). The post hoc test revealed that the group of homozygous I allele carriers was significantly different from the ID and the DD group ($p=0.007$ and 0.001 , respectively). On multiple linear regression controlled for effects of the two other polymorphisms homozygous ACE I allele homozygotes had 109% higher

Table 2 100 mg HCT-induced additional 24-h excretion by genotype

ACE I/D	I/I	I/D	D/D	<i>p</i> value ^a
<i>n</i>	24	53	26	
Volume (ml)	772 (792)	828 (822)	272 (790)	
Na^+ (mmol)	150 (73)	146 (73)	139 (76)	
Cl^- (mmol)	191 (77)	179 (82)	166 (84)	
K^+ (mmol)	44 (21)	33 (27)	16 (26)	0.0006
Ca^{2+} (mmol)	-0.5 (1.1)	-0.4 (1.1)	-0.5 (1.7)	
ADD1 Gly460Trp	Gly/Gly	Gly/Trp	Trp/Trp	
<i>n</i>	73	23	7	
Volume (ml)	717 (871)	695 (712)	168 (729)	
Na^+ (mmol)	152 (73)	125 (69)	140 (86)	
Cl^- (mmol)	187 (81)	154 (75)	165 (94)	
K^+ (mmol)	33 (28)	31 (24)	10 (17)	
Ca^{2+} (mmol)	-0.2 (1.2)	-0.7 (0.8)	-1.7 (2.1)	0.002
GNB3 C825T	C/C	C/T	T/T	
<i>n</i>	47	41	15	
Volume (ml)	745 (713)	602 (997)	655 (721)	
Na^+ (mmol)	156 (65)	140 (79)	122 (79)	
Cl^- (mmol)	188 (73)	177 (87)	151 (87)	
K^+ (mmol)	30 (29)	33 (26)	30 (25)	
Ca^{2+} (mmol)	-0.4 (1.1)	-0.4 (1.6)	-0.5 (0.7)	

^aStatistical difference was tested by linear regression analysis; *p* values are given for $p<0.05$ after Bonferroni adjustment for 15 tests (five parameters with three polymorphisms)

additional K^+ excretion than homozygous D allele carriers. This effect was mainly due to an association of the ACE I/D polymorphism with the induced K^+ excretion in the 6–24 h interval (Fig. 3). With the 25 mg dose comparable trends in the same directions were observed but quantitatively the genotype-related differences were not very strong ($r^2=0.9\%$) and statistically not significant.

The 24-h additional Na^+ , Cl^- and volume excretion induced by 25 and 100 mg HCT was not significantly associated with the ADD1 polymorphism considering adequate adjustment for multiple testing (Table 2). Multiple linear regression analysis controlled for the effects of the two other polymorphisms revealed that homozygote ADD1 Trp allele carriers had 19% less additional Cl^- excretion than homozygous Gly-allele carriers but significant only without adjustment for multiple testing. This effect was mainly due to an association of the ADD1 polymorphism with the induced Cl^- excretion in the 6–24 h interval. The effects on the additional Na^+ excretion were in the same direction and magnitude but not significant. With the 25 mg dose nonsignificant trends in the same direction were observed. There appeared also to be a relation between the HCT-induced potassium excretion and the ADD1 polymorphism (Table 2). However, control for the effect of the ACE I/D polymorphism on the potassium excretion unambiguously showed that the apparent ADD1 effect was only due to the ACE polymorphism.

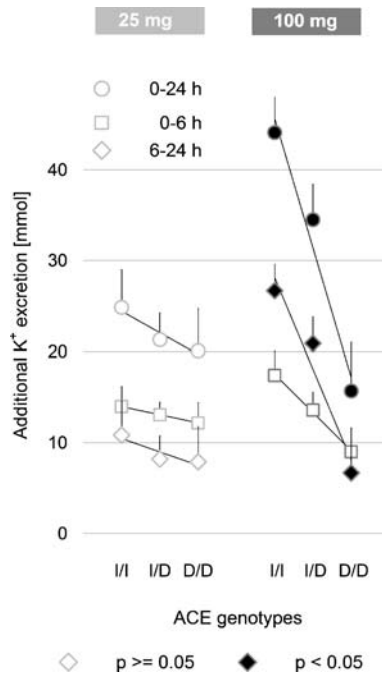


Fig. 3 Interval-wise association of the HCT-induced K^+ excretion with the ACE I/D polymorphism depicting the mean additional (SEM) K^+ excretion induced by 25 mg (light grey symbols) and 100 mg HCT (dark grey symbols) stratified for the ACE I/D genotype; 0–24 h total additional excretion is depicted by circles, 0–6 h additional excretion by squares and 6–24 h excretion by diamonds. $P < 0.05$ is denoted by black filling of the symbols. The p value was 0.0006 and 0.002 for the association with the 100 mg 0–24 h and 6–24 h interval, respectively. Black lines are the respective resulting linear regression lines

The 24-h additional Na^+ , Cl^- and volume excretion induced by 25 and 100 mg HCT was not significantly associated with the GNB3 polymorphism considering adequate adjustment for multiple testing (Table 2). Multiple linear regression analysis controlling for effects of the two other polymorphisms revealed that homozygous GNB3 T allele carriers had 18% less additional Na^+ excretion than homozygous C allele carriers but that was statistically significant only without adjustment for multiple testing. This effect was mainly due to an association of the GNB3 polymorphism with the induced Na^+ excretion in the 6–24 h interval. With the 25 mg dose, comparable trends in the same directions were observed. The additional Cl^- excretion induced by 25 mg and 100 mg HCT was similarly associated with the GNB3 polymorphism but the trends were not significant.

Both HCT doses induced mean additional Ca^{2+} excretion up to the 6–10 h interval. The net effect of Ca^{2+} retention was mainly due to Ca^{2+} retention in the 10–24 h interval. The HCT-induced calcium retention was significantly linked to the ADD1 polymorphism (linear regression $r^2 = 9.4\%$, $p = 0.002$, ANOVA $p = 0.006$, Table 2, Fig. 4). Post hoc tests revealed that the difference between the two groups of homozygotes was significant ($p = 0.003$). Multiple linear regression analysis controlling for the effects of the two other polymorphisms revealed a 4.6-fold higher Ca^{2+} retention after 100 mg HCT in ADD1 Trp allele homozygotes compared to homozygous Gly allele carriers.

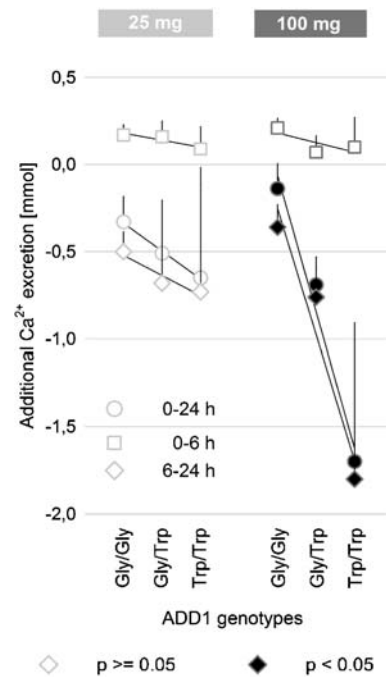


Fig. 4 Interval-wise association of the HCT-induced Ca^{2+} excretion with the ADD1 polymorphism depicting the mean additional (SEM) Ca^{2+} excretion induced by 25 mg (light grey symbols) and 100 mg HCT (dark grey symbols) stratified for the ADD1 Gly460Trp genotype; 0–24 h total additional excretion is depicted by circles, 0–6 h additional excretion by squares and 6–24 h excretion by diamonds. $P < 0.05$ is denoted by black filling of the symbols. The p value was 0.002 and 0.006 for the association with the 100 mg 0–24 h and 6–24 h interval, respectively. Black lines are the respective resulting linear regression lines

The effect was mainly due to an association in the 6–24 h interval (Fig. 4). Comparable effects of minor extent ($r^2 = 0.6\%$) were observed with 25 mg HCT (Fig. 4). Notably, in addition to the stronger calcium retention in the Trp allele carriers, calciuresis was reversed to calcium retention earlier in the Trp allele carriers (median in the 3–6 h interval in Trp/Trp, in the 6–10 h interval in Gly/Trp carriers and the 10–24 h interval in Gly/Gly carriers).

High intraindividual stability in heart rate and blood pressure measures between the 2 placebo days was observed (Table 1, Fig. 2). The doses of 25 mg and 100 mg HCT induced dose-dependent increases in the mean resting heart rate and reduced the mean systolic and diastolic arterial pressure over 5 min standing after the 5-min resting period. Carriers of the ACE insertion allele had a higher resting heart rate than carriers of the deletion allele. This effect was observed on all 4 study days with means between 1.8 and 3.5 bpm per insertion allele at the different study days. Concerning the drug effect, no association of the polymorphisms with any of the measured cardiovascular parameters was observed.

Discussion

This study tested the hypothesis that the ACE I allele, the ADD1 Trp460 allele and the GNB3 T823 allele were

associated with stronger short-term diuretic effects of HCT. In a population of healthy, male volunteers with normal blood pressure we could not observe significantly stronger thiazide-induced excretion of Na^+ , Cl^- or volume with the three alleles associated with stronger blood pressure lowering in hypertensives. In fact, with GNB3 and ADD1 the insignificant trends were even in a direction contrary to the expectations derived from the prior data [14–16, 19]. We observed an association of the ACE I allele with higher thiazide-induced potassium excretion and an association of the ADD1 Trp allele with stronger thiazide-induced calcium retention.

The ACE I allele was associated with higher thiazide-induced potassium excretion in a clear gene-dose relationship. The lower ACE serum activity associated with the I allele may be linked with lower angiotensin II and aldosterone concentrations, less expression of the epithelial Na^+ channel ENaC and less sodium reabsorption via ENaC (Fig. 5). If feedback includes higher expression of the Na^+ - Cl^- cotransporter NCC, I allele carriers would be predisposed to a stronger thiazide effect. This fits exactly with what we found: (1) higher resting heart rate in I allele carriers may indicate less Na^+ reabsorption leading to less blood vessel filling and (2) more than doubled thiazide-induced kaliuresis indicating that the primarily expected higher sodium excretion is covered by Na^+ reabsorption via ENaC with compensatory higher K^+ excretion. The finding of higher kaliuresis with the I allele may have significant epidemiological impact for the safety of diuretic therapy given the long-standing concerns about the hazardous effects of diuretic-induced hypokalaemia [22, 23]. However, the coincidence of the therapeutically desired stronger blood pressure reduction with stronger kaliuresis may explain why thiazide therapy with clinically adjusted doses was not associated with the ACE I/D polymorphism [13]. This entails the hypothesis that I allele carriers may clinically

be adjusted to lower mean doses of thiazide diuretics, but this is a speculation requiring confirmatory studies.

Thiazide diuretics in contrast to loop and potassium-sparing diuretics induce a net retention of calcium [6]. A passive basolateral Na^+ - Ca^{2+} antiport driven by low intracellular Na^+ concentration results in luminal Ca^{2+} influx (Fig. 6) [24]. Both thiazides and the ADD1 Trp allele cause low intracellular Na^+ concentration [15] predisposing Trp allele carriers to higher calcium reabsorption. This may explain why thiazide-induced calcium retention was faster and stronger in carriers of the ADD1 tryptophan allele. It remains to be investigated whether the effect persists on the long term. Calcium retention may help in treating osteoporosis [25–28]. Then Trp allele carriers may benefit more strongly from thiazides than carriers of the glycine allele.

In hypertension (to which the Trp allele predisposes), Trp allele carriers are more salt sensitive and experience stronger blood pressure lowering in response to HCT [16]. Both findings suggest that the ADD1 Trp allele causes more Na^+ reabsorption in consequence of the higher Na^+ - K^+ -ATPase activity induced by the Trp allele [15]. Our finding of no association, if at all with a trend to a lower thiazide response in Trp allele carriers, suggests counter-regulation including downregulation of the NCC. And second, that the Trp allele predisposes to hypertension mainly in combination with an insufficient ability to downregulate the NCC in response to a stronger Na^+ pull.

The GNB3 T allele is associated with downregulated plasma renin concentrations despite unaltered plasma aldosterone concentrations in hypertensives but not in normotensive subjects [29]. Thereby findings that hypertensive T allele carriers experience stronger blood pressure lowering than C allele carriers [19] suggest (1) stronger NCC-mediated NaCl reabsorption in T allele carriers and (2) that this reabsorption is effectively levelled out in normotensives. The latter is in accordance with our results of vir-

ACE insertion allele

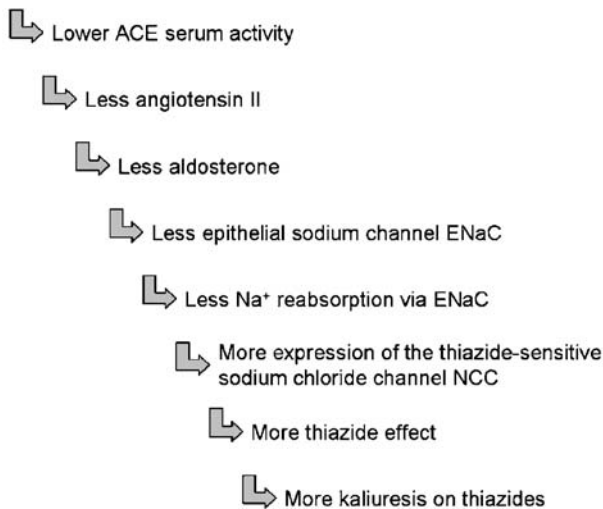


Fig. 5 Putative chain of causation from the ACE I/D genotype to the kaliuretic effect of thiazide diuretics

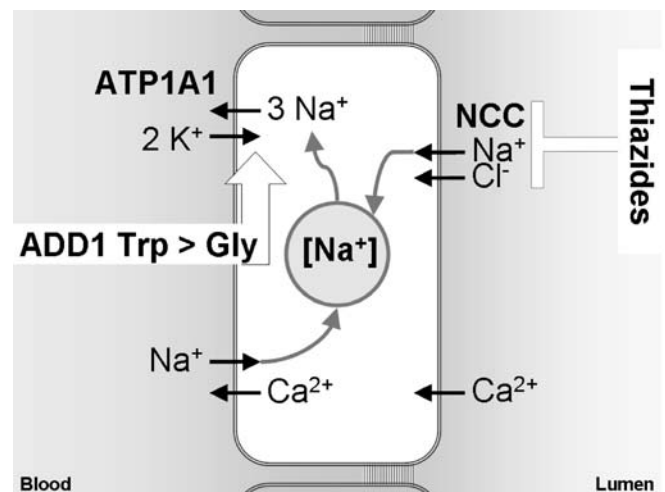


Fig. 6 Putative chain of causation from the ADD1 Gly460Trp genotype to the calcium-sparing effect of thiazides. ADD1 alpha-adducin with the tryptophan (*Trp*) and the glycine (*Gly*) allele, *ATP1A1* the renal form of the Na^+ - K^+ -ATPase, an active 3 Na^+ - 2 K^+ antiporter, *NCC* the passive Na^+ - Cl^- cotransporter

tually no effect. The former may be mediated via a Cl^- pull rather than via a Na^+ pull that should have led to an association with calcium retention in analogy to the ADD1 polymorphism. To provide a Cl^- pull, the T allele may primarily increase the opening of a basolateral KCl cotransporter. A KCl cotransporter expressed in the same nephron part as the NCC and regulated by β -adrenoceptor agonists like KCC4 may be a sensible candidate [30, 31], a hypothesis that needs further investigation. Concerning NCC, our findings suggest that the Trp allele predisposes to hypertension mainly in combination with an insufficient ability to downregulate the NCC in response to a stronger Cl^- pull.

In conclusion, no bimodal or trimodal distribution of any of the tested parameters was observed. In healthy, normotensive volunteers we did not observe the anticipated effects on Na^+ , Cl^- and volume excretion and possible reasons for the discrepancy were discussed above. We observed an association of the ACE I allele with increase potassium excretion and an association of the ADD1 Trp460 allele with stronger calcium excretion. Both findings may contribute to our understanding of the drug-gene interactions and suggest clinical relevance that can now be studied.

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