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Dahlberg, Jonas; Nilsson, Lars-Olof; von Wowern, Fredrik; Melander, Olle

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Polymorphism in NEDD4L Is Associated with Increased Salt Sensitivity, Reduced Levels of P-renin and Increased Levels of Nt-proANP

Jonas Dahlberg*, Lars-Olof Nilsson*, Fredrik von Wowern, Olle Melander

Department of Clinical Sciences, Malmö University Hospital and Lund University, Malmö, Sweden

Objective. Neuronal precursor cell expressed developmentally down-regulated 4-like (NEDD4L) is a regulator of the amiloride-sensitive epithelial sodium channel (ENaC), thus a candidate gene for salt sensitivity. Carriers of an intact NEDD4L C2-domain, encoded by the NEDD4L rs4149601 (G/A) GG genotype, together with the C-allele of the NEDD4L rs2288774 (C/T) polymorphism have previously been shown to have increased blood pressure. Our aim was to test if genetic variation in NEDD4L is associated with increased salt sensitivity. Methods. 39 normotensive subjects were studied. The difference in 24-hour systolic blood pressure after four weeks on 150 mmol/day NaCl intake and four weeks on 50 mmol/day NaCl was defined as salt sensitivity. The rs4149601 and rs2288774 polymorphisms were genotyped using PCR-based techniques. Results. Carriers of the rs4149601 GG-genotype together with the rs2288774 CC-genotype had significantly higher salt sensitivity (median, IQR) (18.0, 7.5–20.0 mmHg vs 6.0, 0.0–10.0 mmHg, P = 0.007) and lower plasma renin concentration (P-renin) (6.0, 2.0–9.5 mU/L vs 15.0, 9.0–24.0 mU/L, P = 0.005) as compared to non-carriers of these genotypes. In carriers of the rs4149601 GG-genotype together with the rs2288774 CC- or CT-genotype, as compared to non-carriers, salt sensitivity was (8.0, 6.0–18.0 mmHg vs 5.0, 0.0–10.0 mmHg, P = 0.07) and P-renin (9.0, 6.0–16.0 mU/L vs 15.0, 9.0–28.0 mU/L, P = 0.03). Conclusion. Genetic NEDD4L variation seems to affect salt sensitivity and P-renin in normotensive subjects, suggesting that genotyping of NEDD4L may be clinically useful in order to identify subjects who benefit from dietary salt restriction in the prevention of hypertension.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular morbidity and mortality and affects 27% of the adult Swedish population [1]. Blood pressure has a normal distribution in the population suggesting a multifactorial background of blood pressure regulation and hypertension development. Genetic factors might explain 30–40% of population blood pressure variation [2] and the genetic component is most likely composed of many individual genetic variants. Environmental risk factors such as stressful lifestyle, lack of physical exercise, high caloric intake and salt-rich diet also contribute to elevated blood pressure and development of hypertension. Furthermore, the environmental risk factors are likely to interact with the genetic susceptibility variants. Given the complex etiology and pathophysiology of hypertension, the search for genetic variants of importance for blood pressure has been suggested to be more comprehensible by dissecting an “intermediate phenotype” of blood pressure such as salt sensitivity. Such a phenotype is plausibly influenced by substantially fewer genes and environmental factors [3]. Salt sensitivity, i.e. the individual blood pressure reaction to a given change in salt intake is a continuous trait [4] and cut-off limits used to dichotomize the trait are arbitrary and have differed between studies. The heritability of systolic salt sensitivity calculated in Black pedigrees has been shown to be as high as 74% suggesting a strong genetic component of this trait [5]. In addition, hypertensive patients are more salt sensitive than normotensive subjects [4] and normotensive subjects with heredity for hypertension have increased salt sensitivity [6], indicating that salt sensitivity is partially related to an inherited predisposition for hypertension.

Salt restriction at the population level, with the aim to reduce population blood pressure, is difficult to achieve [7,8] and its ability to lower the incidence of cardiovascular disease is controversial. However, identifying and intervening with salt restriction in the most salt sensitive segment of the population would probably be a very cost-effective way of treating and preventing hypertension in this particular subset of the population. In a clinical context it is cumbersome to measure salt sensitivity. It therefore seems essential to identify bio-markers for salt sensitivity and thus provide physicians a manageable tool of whom to give intense educational dietary advice. We have previously shown that salt sensitivity, defined as the difference between blood pressure after one week of high (240 mmol daily) and one week of low (10 mmol daily) salt intake is directly correlated to N-terminal atrial naturetic peptide (Nt-proANP) and inversely correlated to plasma renin activity [9,10]. In a study recently completed, “Salt Reduction to...
Avoid Hypertension” (SARAH), we found that salt sensitivity defined as the difference between blood pressure after four weeks of high (150 mmol daily) and four weeks of low (50 mmol daily) salt intake, i.e. a more clinically relevant intervention, was directly correlated to NT-proANP and inversely correlated to plasma concentration of renin (P-renin) [11]. These studies thus suggest that renin and NT-proANP may be used to predict salt sensitivity.

It has been proposed that salt sensitivity is related to the inability of the kidney to excrete sodium [12,13]. Most monogenic forms of human hypertension are caused by mutations increasing renal sodium reabsorption primarily at the level of the amiloride sensitive sodium channel (ENaC) [14,15]. These rare forms of hypertension are further characterized by extreme salt sensitivity and suppressed P-renin. Although salt sensitivity and the accompanying P-renin suppression we have observed at the population level is usually far from as extreme as that seen in monogenic forms of hypertension, the clinical resemblance between them suggests that salt sensitivity may have partially similar pathophysiology as monogenic forms of hypertension. In one of the monogenic forms of hypertension, Liddle’s syndrome, activating ENaC mutations affect the PY motif of ENaC with the consequence that ENaC becomes insensitive to down regulation by a protein coded by the Neural precursor cell Expressed Developmentally Down-regulated 4 Like (NEDD4L) gene [16,17]. Normally the NEDD4L protein interacts with the PY motif and regulates the cell surface expression of ENaC by ubiquitination, thereby affecting the rate of sodium reabsorption in the distal nephron.

We recently showed that a combination of two common single nucleotide polymorphisms (SNPs) (rs4149601 and rs2288774) located in the NEDD4L gene is associated with blood pressure variation in a population study from Malmo, Sweden [10]. The G→A substitution at the rs4149601 polymorphism at the first nucleotide of exon 1 of the NEDD4L gene leads to an alternate splice site, which generates a transcript encoding a protein lacking the functionally crucial Ca2+-dependent lipid binding domain (C2 domain) [19]. NEDD4L and its paralog NEDD4 lacking the C2 domain, down-regulate ENaC more potently than NEDD4L with an intact C2 domain [16,20–22] suggesting that carriers of the G-allele had higher ENaC expression and sodium reabsorption in the distal nephron.

The protocol of the study was approved by the ethics committee of Lunds University, and all study participants gave written informed consent. The procedures were in accordance with institutional guidelines.

Subjects
Forty-six subjects with no history of hypertension, diabetes or kidney diseases were recruited through newspaper advertisements. Of these, 7 did not complete the study because of infections with fever (n = 2) or refusal to take the study capsules regularly (n = 5). Thus, 39 healthy subjects (53±11 years of age, BMI 26.3±3.1 kg/m², number of men/women 20/19) completed the study. Other phenotype characteristics are presented in Table 1.

Design
All the subjects were first examined at baseline, i.e. with the subjects on their regular diets before the standardization of salt intake started. During a period of eight weeks the subjects were given all their meals and drinks from a metabolic ward. Energy intake was adjusted according to body weight and gender (2000–2600 kcal/day). Apart from the provided meals and drinks, subjects were prohibited to ingest anything else apart from tap water. The diet during the eight weeks of study contained 50 mmol of salt (NaCl) daily. On top of this, the study participants were given either 100 mmol of salt in capsules (totally 150 mmol of salt daily) for four weeks and corresponding number of placebo capsules (totally 50 mmol of salt daily) for four weeks in a random order double blind crossover design [11].

Ambulatory blood pressure (ABP) and 24-hour urinary excretion of sodium were measured at baseline, after the four weeks on the high salt intake (150 mmol daily) and after the four weeks of low salt intake (50 mmol daily). ABP was measured using an ABPM 90207 device (Spacelabs Medical Inc, Redmond, WA, USA), which was applied on their left arm using appropriate cuff sizes according to arm circumference. During the daytime period (6 am–10 pm) blood pressure was recorded every 20 min and during the nighttime period (10 pm–6 am) every 60 min. Subjects were advised to relax the arm and keep it along the body during blood pressure measurements. Urine was collected during the same 24-hour as the ABP measurements.

Salt sensitivity was defined as the difference between 24-hour systolic ABP after the four weeks on high salt intake and 24-hour systolic ABP after the 4 weeks on low salt intake. In addition, the salt induced change in diastolic ABP (diastolic salt sensitivity) was recorded and systolic and diastolic salt sensitivity were further divided into the daytime and nighttime periods. P-renin and NT-proANP were measured at baseline in the upright position without prior rest using RIA diagnostic kits as described previously [9,10]. Urine and serum concentrations of sodium, potassium and creatinine were measured by standard biochemical methods at baseline, after the high- and low salt intake periods.

Methods
DNA was extracted from venous whole blood by standard methods [23]. Two variants in the NEDD4L gene (rs4149601, G→A in exon 1 and rs2288774, T→C, in intron 6) were genotyped using PCR. The polymorphisms were analysed using forward primer 5′-GCTTTCCTCATTGACTAAACCTTTTAAATTATGTG-3′ and reverse primer 5′-GGTAAAGACTTTGGCTTTGGG-3′ (rs4149601) and forward primer 5′-CAAGTGCTCAGTGTGTTTGAAGCT-3′ and reverse primer 5′-AGAAGGCTGAATGAGAGCGCT-3′ (rs2288774) synthesized by Applied Biosystems (Applied Biosystems, Foster City, California, USA). TaqMan MGB probes were custom synthesized by Applied Biosystems: (rs4149601, G) FAM-ATTGAGCAAGTACAC,
Statistics

All data in the study was analyzed with SPSS statistical software (version 11.5, SPSS Inc., Chicago, Illinois, USA). Data is presented as mean ± standard deviation (SD) for normally distributed variables and as medians and interquartile ranges (IQR) for variables that were not normally distributed. Significance of group-wise and pair-wise differences in continuous variables was tested with t-test/paired t-test or Mann-Whitney/Wilcoxon signed rank test depending on whether data was normally distributed or not. P<0.05 was considered statistically significant.

RESULTS

The rs4149601 (exon 1) and rs2288774 (intron 6) polymorphisms were tested separately and in combination for association to salt sensitivity indices and to baseline levels of Nt-proANP and P-renin.

Carriers of the rs4149601 GG-genotype had significantly lower P-renin than carriers of the rs4149601 GA or AA genotypes whereas no other phenotype differed significantly by genotype (Table 2). There was no association between any of the phenotypes and the rs2288774 polymorphism (Table 2).

Carriers of the rs4149601 GG-genotype together with the rs2288774 CC-genotype had significantly higher 24-hour, daytime and nighttime systolic salt sensitivity, lower P-renin and higher Nt-proANP compared with subjects who do not carry this genotype combination of the NEDD4L gene.

DISCUSSION

The key finding of this study is that the carriers of the rs4149601 GG-genotype together with rs2288774 CC-genotype have enhanced systolic salt sensitivity, lower P-renin and higher Nt-proANP compared with subjects who do not carry this genotype combination of the NEDD4L gene.

NEDD4L and its paralog NEDD4 lacking the functionally crucial C2-domain down-regulate ENaC more potently than the protein variants with intact C2-domain [19,20]. As the rs4149601 A-allele alters a splice site leading to preferential deletion of the C2 domain [21], subjects carrying the G-allele would be expected to have a less efficient NEDD4L induced down-regulation of ENaC and thus increased renal sodium reabsorption. Interestingly, carriers of the rs4149601 GG-genotype had significantly lower P-renin but did not differ regarding salt sensitivity (Table 2), indicating reduced activity of the renin-angiotensin-aldosterone system (RAAS) as a response to a slight increase in renal sodium reabsorption in order to counterbalance the effect of the G-genotype on salt sensitivity. Carrier ship of the rs2288774 CC-genotype seemed to amplify the effect of the rs4149601, thereby unmasking clinically detectable salt sensitivity. In contrast to the rs4149601, the functional consequence of the rs2288774 at the molecular level is unknown. The NEDD4L gene is highly polymorphic suggesting that the rs2288774 polymorphism in intron 6 could be non-functional but be in linkage disequilibrium with another functional NEDD4L variant. Interestingly, intronic SNPs in the transcription factor 7-like 2 (TCF7L2) gene have been strongly associated with another complex phenotype, that of type 2 diabetes.
type 2 diabetes mellitus, in numerous populations of various ethnicities [25]. Thus, another possibility is that rs2288774 is functional, despite being intronic. Such functional mechanisms may involve interference with intronic transcription factor binding sites, leading to altered NEDD4L expression, or introduction of a cryptic splice site. Importantly, in a recent study on genetic NEDD4L variance and blood pressure in subjects without antihypertensive treatment, we found that the rs4149601 polymorphism seems to interact with another NEDD4L polymorphism, rs2288774 [18]. In that study carriers of the rs4149601 GG-genotype together with rs2288774 CT or CC-genotypes had approximately 2/1 mmHg higher office blood pressure compared to non-carriers [18]. Carriers of the same genotype combination in the present study tended to have higher systolic salt sensitivity and had significantly lower P-renin (Table 3). On the other hand, carriers of the rs4149601 GG-genotype together with rs2288774 CC-genotype, who had the greatest systolic salt sensitivity in the present study (Table 3), did not have significantly higher blood pressure in the previous population study [18]. Although related, it is important to stress that blood pressure and salt sensitivity are different phenotypes. The relatively greater suppression of baseline P-renin in carriers of the rs4149601 GG-genotype together with rs2288774 CC-genotype compared to that seen in carriers of rs4149601 GG-genotype together with rs2288774 CT or CC-genotypes (Table 3) may level out the effect on blood pressure through RAAS-dependent mechanisms other than those directly affecting renal sodium reabsorption. Despite the substantially smaller material used in the present study, as compared with the previous population study on blood pressure, there were several reasons to believe that we would have power enough to detect an effect of the NEDD4L polymorphisms on salt sensitivity. Most importantly, salt sensitivity is a much less complex phenotype than blood pressure. Blood pressure is influenced by numerous environmental factors such as stress, physical activity, caloric intake and level of salt intake. Although salt sensitivity may also be affected by a number of environmental factors, the standardized phenotype of salt sensitivity used in the present study is primarily influenced by the shift of salt intake from 150 mmol to 50 mmol daily and the individual inherited and acquired capacity of handling changes in salt intake. As fewer environmental factors are implicated in salt sensitivity than in blood pressure, it can be speculated that fewer genes conferring gene-environment interactions are involved in salt sensitivity. In addition, whereas heritability of blood pressure is usually around 30–40%, that of systolic salt sensitivity has been reported to be as high as 74% [5], suggesting a substantially greater genetic component of systolic salt sensitivity than for blood pressure. Finally, in our previous study on blood pressure, the phenotype used was office blood pressure, whereas in the present study of salt sensitivity the more accurate blood pressure phenotype of ABP was used to define blood pressure after the two 24/14-hour systolic blood pressure; Δ24SBP, delta 24-hour diastolic blood pressure; Δ24DBP, delta 24-hour diastolic blood pressure; ΔDBP, delta diastolic blood pressure; Δ24SBP, delta diastolic blood pressure; ΔNSBP, delta night-time systolic blood pressure; ΔNDDBP, delta night-time diastolic blood pressure. P-value; CC vs CT or TT

Table 2. Salt-induced changes in blood pressure and baseline P-renin and Nt-proANP in different genotype carriers.

| rs4149601 genotype variants | GG (n = 16) | GA (n = 20) | AA (n = 3) | P-value; GG vs GA or AA |
|-----------------------------|------------|------------|-----------|------------------------|
| Δ24SBP(mmHg)               | 6.5(3.0–13) | 6.5(0.0–10) | 2.0(–0.8–15) | 0.315                  |
| Δ24DBP(mmHg)               | 1.5(–1.0–9.75) | 3.5(–0.5–5.75) | =1.0(–5.0–8.0) | 0.703                  |
| ΔDBSBP(mmHg)               | 6.5(2.0–14.5) | 6.0(–2.0–9.0) | 6.0(–6.0–7.0) | 0.343                  |
| ΔDBBP(mmHg)                | 2.0(–3.0–9.75) | 2.0(–1.0–4.75) | 1.0(–5.0–3.0) | 0.921                  |
| ΔNSBP(mmHg)                | 11.5(2.25–15) | 6.0(0.25–9.0) | 3.0(–15–30) | 0.107                  |
| ΔNDDBP(mmHg)               | 4.5(1.25–9.5) | 4.0(–2.75–7.0) | 4.0(–8.0–16.0) | 0.196                  |
| P-renin(mU/L)              | 10.5(6.5–15.8) | 18.0(10.0–28.0) | 9.0(2.0–25.0) | 0.045                  |
| Nt-proANP(pmol/L)          | 510(419–803) | 510(422–646) | 620(440–786) | 0.641                  |

| rs2288774 genotype variants | CC (n = 12) | CT (n = 14) | TT (n = 13) | P-value; CC vs CT or TT |
|-----------------------------|------------|------------|-----------|------------------------|
| Δ24SBP(mmHg)                | 7.5(3.0–16.5) | 4.0(–1.25–7.75) | 7.0(2.5–12) | 0.168                  |
| Δ24DBP(mmHg)                | 4.5(–0.5–8.25) | 1.0(–2.0–4.0) | 3.0(–1.0–6.5) | 0.221                  |
| ΔDBSBP(mmHg)                | 7.5(2.75–19) | 3.5(–2.0–7.0) | 7.0(0.5–11.0) | 0.178                  |
| ΔDBBP(mmHg)                 | 3.0(0.25–9.75) | –0.5(–1.5–4.0) | 3.0(–2.0–5.5) | 0.188                  |
| ΔNSBP(mmHg)                 | 9.0(0.25–15) | 4.5(–1.0–8.75) | 9.0(3.5–12.5) | 0.443                  |
| ΔNDDBP(mmHg)                | 5.5(–2.75–8.0) | 3.5(–2.25–7.0) | 5.0(2.0–7.5) | 0.845                  |
| P-renin(mU/L)               | 10.5(6.5–19.5) | 14.0(9.0–21.5) | 14.5(6.75–28.75) | 0.185                  |
| Nt-proANP(pmol/L)           | 659(438–844) | 559(359–732) | 489(436–599) | 0.150                  |

Blood pressure change in low versus (vs) high salt intake; Δ24SBP, delta 24-hour systolic blood pressure; Δ24DBP, delta 24-hour diastolic blood pressure; ΔDBSBP, delta daytime systolic blood pressure; ΔDBBP, delta daytime diastolic blood pressure; ΔNSBP, delta night-time systolic blood pressure; ΔNDDBP, delta night-time diastolic blood pressure. P-value; <0.05 is considered statistically significant. Numbers represent Median(Inter Quartile Range).

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which over-expression of ENaC in the distal nephron leads to hyper-
reabsorption of sodium, volume expansion, suppression of RAAS
and extremely salt sensitive hypertension. Thus, the associations
between the NEDD4L rs4149601 and rs2288774 polymorphisms
on the one hand and P-renin and Nt-proANP on the other, are likely
to be secondary to genetically mediated enhancement of tubular
sodium reabsorption rather than being a direct effect of the genetic
variants on renin and Nt-proANP secretion.

Our associations were generally stronger for systolic than for
diastolic salt sensitivity (Tables 2 and 3). This is probably explained
by the fact that the heritability of systolic salt sensitivity is
substantially higher than for diastolic salt sensitivity [5] giving us
a greater power to detect genetic factors influencing systolic salt
sensitivity.

In summary, we show that carriers of the rs4149601 GG-
genotype together with rs2288774 CC-genotype of the NEDD4L
gene have enhanced systolic salt sensitivity, lower P-renin and
higher Nt-proANP. Our data suggests that carriers of this
genotype combination have increased renal sodium reabsorption
through reduced NEDD4L induced down-regulation of ENaC. As
salt sensitivity differs a lot between individuals, genotyping of these
genic NEDD4L markers may be a clinically useful tool in
identifying those individuals who would gain the greatest blood
pressure lowering benefit from reduced dietary salt intake.

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Author Contributions

Conceived and designed the experiments: OM JD LN. Performed the
experiments: OM JD LN. Analyzed the data: OM JD LN Fv. Contributed
reagents/materials/analysis tools: OM. Wrote the paper: OM JD LN Fv.

REFERENCES

1. SBU (2004) Moderately Elevated Blood Pressure. The Swedish council on
Technology Assessment in Health Care.

2. Fava C, Burri P, Ahmorgen P, Groop L, Hulten U, et al. (2004) Heritability of
ambulatory and office blood pressure phenotypes in Swedish families.
J Hypertens 22: 1717–1721.

3. Melander O (2001) Genetic factors in hypertension–what is known and what
does it mean? Blood Press 10: 254–270.

4. Weinberger MH, Miller JZ, Luft FC, Grun CE, Fineberg NS (1986) Definitions
and characteristics of systolic sensitivity and blood pressure resistance.
Hypertension 8: 1127–134.

5. Sverkey LP, McKeown SP, Wilson AF (1996) Heritability of salt sensitivity in
black Americans. Hypertension 28: 434–438.

6. Sharma AM, Schorr U (1996) Salt sensitivity and insulin resistance: Is there
a link? Blood Press Suppl 1: 59–63.

7. Graudal NA, Galloe AM, Garred P (1998) Effects of sodium restriction on blood
pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride:
a meta-analysis. Jama 279: 1303–1391.

8. He FJ, MacGregor GA (2002) Effect of modest salt reduction on blood pressure:
a meta-analysis of randomized trials. Implications for public health. J Hum
Hypertens 16: 761–770.

9. Melander O, Frandsen E, Groop L, Hulten U (2002) Plasma ProANP(1-30)
reflects salt sensitivity in subjects with heredity for hypertension. Hypertension
39: 996–999.

10. Melander O, Frandsen E, Groop L, Hulten U (2003) No evidence of a relation
between 11beta-hydroxysteroid dehydrogenase type 2 activity and salt
sensitivity. Am J Hypertens 16: 739–744.

11. Melander O, von Worthern F, Frandsen E, Burri P, Willemsen G, et al. (2007)
Moderate salt restriction effectively lowers blood pressure and degree of salt
sensitivity is related to baseline concentration of renin and N-terminal atrial
natriuretic peptide in plasma. J Hypertens 25: 619–627.

12. Guyton AC (1987) Renal function curve–a key to understanding the
pathogenesis of hypertension. Hypertension 10: 1–6.

13. Guyton AC (1989) Dominant role of the kidneys and accessory role of whole-body
autoregulation in the pathogenesis of hypertension. Am J Hypertens 2: 573–585.

Table 3. Salt-induced changes in blood pressure, and baseline
P-renin and Nt-proANP in different genotype carriers.

| rs4149601 GG-rs2288774 CC/CT genotype variants vs non-carriers | GG-CC/CT (n = 11) | Non-carriers (n = 28) | P-value |
|---------------------------------------------------------------|-------------------|----------------------|---------|
| Δ24SBP (mmHg)                                                | 8.0(6.0–18)       | 5.0(0.0–10)          | 0.067   |
| Δ24DBP (mmHg)                                                | 2.0(1.0–11)       | 2.0(1.0–5.75)        | 0.158   |
| ΔDSBP (mmHg)                                                 | 8.0(4.0–19)       | 5.0(2.0–8.75)        | 0.058   |
| ΔDDBP (mmHg)                                                 | 4.0(3.0–11)       | 1.5(1.0–3.75)        | 0.140   |
| ΔNSBP (mmHg)                                                 | 14.0(2.0–15)      | 5.5(0.0–9.0)         | 0.083   |
| ΔNDBP (mmHg)                                                 | 8.0(1.0–10)       | 4.0(2.75–7.0)        | 0.124   |
| P-renin (mU/L)                                                | 9.06(0.16)        | 15.0(9.0–28.0)       | 0.030   |
| Nt-proANP (pmol/L)                                           | 75.4(426–880)     | 493(422–622)         | 0.109   |

| rs4149601 GG-rs2288774 CC genotype vs non-carriers | GG-CC (n = 5) Non-carriers (n = 34) | P-value |
|---------------------------------------------------------------|-------------------|---------|
| Δ24SBP (mmHg)                                                | 18.7(5.0–20)      | 6.0(0.0–10)       | 0.007   |
| Δ24DBP (mmHg)                                                | 9.0(5.0–13.5)     | 1.5(1.25–6.0)      | 0.081   |
| ΔDSBP (mmHg)                                                 | 19.0(5.0–21.5)    | 5.0(2.0–9.0)       | 0.024   |
| ΔDDBP (mmHg)                                                 | 9.0(5.0–13.5)     | 1.5(1.0–4.0)       | 0.117   |
| ΔNSBP (mmHg)                                                 | 15.0(12.5–20)     | 5.0(2.5–9.5)       | 0.001   |
| ΔNDBP (mmHg)                                                 | 8.0(4.5–11)       | 4.0(2.75–7.0)      | 0.089   |
| P-renin (mU/L)                                                | 6.0(2.0–9.5)      | 15.0(9.0–24.0)     | 0.005   |
| Nt-proANP (pmol/L)                                           | 806(590–1186)     | 493(417–655)       | 0.031   |

Blood pressure change in low versus (vs) high salt intake: Δ24SBP, delta 24-hour systolic blood pressure; Δ24DBP, delta 24-hour diastolic blood pressure; ΔDSBP, delta daytime systolic blood pressure; ΔDDBP, delta daytime diastolic blood pressure; ΔNSBP, delta night-time systolic blood pressure; ΔNDBP, delta night-time diastolic blood pressure. P-value<0.05 is considered statistically significant. Numbers represent Median(Inter Quartile Range).

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Figure 1. Salt induced change in 24 hour systolic ABP (salt sensitivity) in the carriers of the rs4149601 GG-genotype together with the rs2288774 CC-genotype vs. all other genotype combinations. Box plot presented as median and inter quartile range. doi:10.1371/journal.pone.0000432.g001

Box plot presented as median and inter quartile range.
14. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, et al. (1994) Liddle’s syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 79: 407–414.

15. Mune T, Rogerson FM, Nakkila H, Agarwal AK, White PC (1995) Human hypertension caused by mutations in the kidney isoform of 11 beta-hydroxysteroid dehydrogenase. Nat Genet 10: 394–399.

16. Kamynina E, Debonneville C, Bens M, Vandewalle A, Staub O (2001) A novel mouse Nedd4 protein suppresses the activity of the epithelial Na+ channel. Faseb J 15: 204–214.

17. Harvey KF, Dinudom A, Cook DI, Kumar S (2001) The Nedd4-like protein KIAA0439 is a potential regulator of the epithelial sodium channel. J Biol Chem 276: 8597–8601.

18. Fava C, von Wowern F, Berglund G, Carlson J, Hedblad B, et al. (2006) 24-h ambulatory blood pressure is linked to chromosome 18q21-22 and genetic variation of NEDD4L associates with cross-sectional and longitudinal blood pressure in Swedes. Kidney Int 70: 562–569.

19. Dunn DM, Ishigami T, Pankow J, von Niederhausern A, Alder J, et al. (2002) Common variant of human NEDD4L activates a cryptic splice site to form a frameshifted transcript. J Hum Genet 47: 665–676.

20. Plant PJ, Yeger H, Staab O, Howard P, Rotin D (1997) The C2 domain of the ubiquitin protein ligase Nedd4 mediates Ca2+-dependent plasma membrane localization. J Biol Chem 272: 32329–32336.

21. Plant PJ, Lafont F, Locat S, Verkade P, Simons K, et al. (2000) Apical membrane targeting of Nedd4 is mediated by an association of its C2 domain with annexin XIIIb. J Cell Biol 149: 1473–1484.

22. Kamynina E, Tume C, Staub O (2001) Distinct characteristics of two human Nedd4 proteins with respect to epithelial Na+ channel regulation. Am J Physiol Renal Physiol 281: F669–677.

23. Vandenplas S, Wild I, Grobler-Rabie A, Brehner K, Ricketts M, et al. (1984) Blot hybridisation analysis of genomic DNA. J Med Genet 21: 164–172.

24. Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 14: 143–149.

25. Zeggini E, McCarthy MI (2007) TCF7L2: the biggest story in diabetes genetics since HLA? Diabetologia 50: 1–4.