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Alpha-adducin 1 (rs4961) gene and its expression associated with sodium sensitivity in hypertensive patients: a cohort study in the western Ukrainian population

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Objective. The aim of this study was to evaluate the association of the α-adducin-1 gene (*ADD1*) (Gly460Trp [rs4961]) polymorphism and its expression in association with renal dysfunction and sodium sensitivity in hypertensive patients in western Ukrainian population.

Methods. One-hundred patients with essential arterial hypertension (EAH) and hypertensive-mediated target organ damage (stage 2), moderate, high, and very high cardiovascular risk were enrolled in case-control study. Sixty healthy individuals were assigned as controls. Sodium sensitivity and sodium resistance were determined by salt load reaction. The *ADD1* (rs4961) genotyping was performed in RT-PCR.

Results. The expression of the quantitative trait loci (eQTL) of *ADD1* gene (rs4961) (chr4:2906707 [hg19]) was confirmed in 37 tissues and organs with 23 phenotypic traits. Two hundred eQTL associations revealed - all cis-variants (cis-QTL); 73 methylation QTL (mQTL), 34 splicing QTL (sQTL), 14 histone modification QTL (hQTL), 2 protein QTL (pQTL), 23 transcript utilization QTL (tuQTL), and 4 loci of incorporated long noncoding areas of RNA (lncRNA). GG-genotype unreliably enhances EAH risk (OR=1.92; 95%CI: 0.90–4.10; p=0.066). Sodium sensitivity was observed in 54.0% of patients and in 20.0% of controls (c2=17.89; p<0.001). Sodium sensitivity in T-allele carriers of the *ADD1* gene (1378G>T; rs4961) dominated 12-fold in general (OR 95%CI: 2.24–64.29; p=0.001), in women – 4.71 times (OR 95%CI: 1.92–11.56; p<0.001), and in men – 4.09 times (OR 95%CI: 1.03–16.28; p=0.041). Sodium sensitivity elevated the likelihood of severe EAH twice (OR=2.19; OR 95%CI: 1.00–5.05; p=0.049).

Conclusion. T-allele associates with sodium sensitivity in essential arterial hypertension patients and increases the risk of hypertension regardless the gender. Sodium sensitivity enhances the probability of severe essential arterial hypertension in observed population.

Keywords: arterial hypertension, alpha-adducin (rs4961) gene, sodium sensitivity, sodium resistance, chronic kidney disease, metabolism, genes expression, transcriptional activity

Arterial hypertension is a multifaceted disease influenced by various genetic, physiological, and environmental factors. Both primary (essential) arterial hypertension (EAH) and secondary arterial hypertension (renal, endocrine, etc.) exhibit a variety of potential molecular mechanisms, pathophysiological, and clinical patterns, and responses to treatment making them heterogeneous conditions.

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Additionally, a complex network of feedback loops involving renal, hormonal, hemodynamic, and nervous systems, which regulate blood pressure (BP), complicates the differentiation between primary and secondary mechanisms of arterial hypertension. Up to date, pathophysiology suggests that no form of hypertension can persist without further renal defect (Ferrari and Bianchi 1997; Guyton and Hall 2000; Dorrington and Pandit 2009). Typically, a BP elevation, regardless of the cause, leads to an increased natriuresis (renal sodium output) to restore and sustain the original condition. Vice versa, hypertension may develop when kidney's sodium excretion is compromised. Consequently, nearly all monogenic hypertension types are associated with sodium metabolism disruptions (Canadas-Garre et al. 2019). Despite recent advances in genome-wide-association studies (GWAS) suggesting several genetic loci linked to renal function traits (Hishida et al. 2018; Sydorchuk et al. 2020; Tavakolidakhrabadi et al. 2024), the most evinced is molecular mechanism of α-adducin gene mutation (Gly460Trp polymorphism), which influence the sodium transport across the renal cell membranes and increase renal tubular sodium reabsorption potentially causing a sodium sensitiveness growth in BP (Li 2012).

Multiple studies have explored the connection between the α-adducin gene mutation and sodium-sensitive hypertension, generally confirming that the 460Trp variant is associated with higher sodium sensitivity (Grant et al. 2002; Zhang et al. 2019, 2022). However, whereas many researches have examined the relationship between α-adducin variants and BP response to sodium, much less is known about the effects of these variants on renal hemodynamics and function. This is noteworthy, given the crucial role of the kidney and its blood supply in regulating sodium homeostasis. Therefore, this gap in knowledge prompted us to investigate the impact of the rs4961 (1378G>T, Gly460Trp) polymorphism of the α-adducin gene (*ADD1*) and its expression on renal dysfunction and association with sodium sensitivity in hypertensive patients in western Ukrainian population (Northern Bukovina).

Material and Methods

Subjects. Patients with EAH were selected based on the acting guidelines and recommendations of the National and European Societies of Cardiology and Hypertension (Williams et al. 2018; Mancia et al. 2023). The research protocol was approved by the Bioethics commission at Bukovinian State Medical University (Protocol №2 from October 19, 2023). All participants signed informed consent to participate in the study. A total of 100 patients with hypertension-mediated organ damage (II stage), 1st–3rd degrees of BP elevation, moderate, high, and very high cardiovascular risk were screened and selected for further investigation.

Inclusion and exclusion criteria are detailed in the previous publications (Sydorchuk et al. 2022, 2023a, b). The patients' age varied from 45 to 70 years (mean 59.87±7.98 years); 21.0% of them were men, 79.0% were women. The control group included 60 practically healthy individuals: 22 men (36.67%), 38 women (63.33%), aged 44.39±5.92 years ($p<0.001$). The groups did not differ by sex.

Laboratory data. Comprehensive examination included: general clinical tests, anthropometry, laboratory tests (general blood and urine tests, fasting plasma glucose, bilirubin, lipid spectrum, serum creatinine, Cystatin-C), instrumental (12-lead ECG, Echocardiogram, office BP, kidneys ultrasound), consultations of an ophthalmologist, and a neurologist in case of need.

Chronic kidney disease (CKD) was diagnosed in 43 patients with EAH according to the recommendations of the US National Kidney Association "Kidney disease improving global outcomes" (KDIGO 2024). Glomerular filtration rate (eGFR) was estimated using the CKD-EPI equation based on Cystatin-C (cys) and creatinine (cr) serum values adjusted for sex. Among the EAH patients with CKD, there were 44.30% women (n=35) and 38.10% men (n=8). A decrease in GFR was defined as ≤60 ml/min/1.73 m² for ≥3 months with or without other signs of renal damage according to the KDIGO 2024 recommendations.

Sodium sensitivity and sodium resistance were determined by salt load reaction method (Weinberger 1996).

Genotyping of rs4961 (1378G>T) polymorphism. The 378G>T, rs4961 SNP (National Center for Biotechnology Information, 2022) of *ADD1* gene was studied in real time qualitative polymerase chain reaction (RT-PCR). The genetic polymorphism study was conducted for 72 EAH patients and 48 subjects of the control group. Lymphocytic DNA was isolated and purified from EDTA-stabilized peripheral venous blood lymphocytes according to the manufacturer's instructions (Thermo Fisher Scientific, USA). Amplification and genotyping were performed on a CFX96 Touch™ device (Bio-Rad Laboratories Inc., USA) using specific complementary TaqMan probes. Protocol of genotyping is detailed in our previous publications (Sydorchuk et al. 2022, 2023a, b). The

CFX96 software recorded the melting temperature of the TaqMan probes taking into account the Fam and Hex fluorescent labels.

Statistical analysis. Statistical processing was performed using the StatSoft Statistica v.7.0 software (StatSoft Inc., USA). Compliance of the genotype distribution with the Hardy-Weinberg equilibrium in the examined population was checked using the c^2 test. Potential risk factors were determined by clinical epidemiology methods (multivariate logistic regression model) using relative risk (RelR), odds ratio (OR) and 95% confidence interval (95% CI) based on the c^2 criterion. Differences were considered significant at p-value <0.05.

Results

Two hundred and forty alleles of the *ADD1* gene (rs4961) were isolated from the blood of 120 subjects (72 EAH patients and 48 practically healthy): 144 alleles of the study group and 96 alleles of the control group. The wild G-allele carriers prevailed over the T-allele 5.26 times in patients (c^2=133.39; p<0.001) and 2.84 times in controls (c^2=44.08; p<0.001) (Table 1). The genotypes distribution did not differ between groups. The allelic distribution showed that the mutated T-allele is by 10.07% more common in the control group compared to EAH patients, conversely the wild G-allele is more common in EAH compared to control (c^2=3.65; p=0.041).

The allelic distribution does not deviate from the *Hardy-Weinberg* equilibrium. Allele frequencies correspond to the following in Caucasian populations: P_G=0.74–0.84, P_T=0.16–0.26 (p>0.05).

In order to analyze the tissue/organ-specific transcriptional activity of the *ADD1* gene, we searched for expression quantitative trait loci (eQTL) for SNP rs4961 using the online QTLbase database. The strongest eQTL of the *ADD1* gene (rs4961) is found in arteries (aorta, tibial, and coronary), esophagus, colon, brain structures, central and peripheral nervous system, uterus, ovaries, vagina, adipose

Table 1
Allelic distribution of *ADD1* gene (1378G>T, rs4961) in hypertensive patients

Genotypes and alleles of *ADD1* gene		Patients n=72 (%)	Control n=48 (%)	χ^2	p-value
ADD1 (1378G>T) n (%)	TT	1 (1.39)	3 (6.25)	–	0.175
	GT	21 (29.17)	19 (39.58)	1.41	0.235
	GG	50 (69.44)	26 (54.17)	2.89	0.089
χ^2; p		χ^2=4.04; DF=2; p=0.133		–	–
ADD1 (1378G>T) n (%)	*T*-allele	23 (15.97)	25 (26.04)	3.65	0.041
	G-allele	121 (84.03)	71 (73.96)		

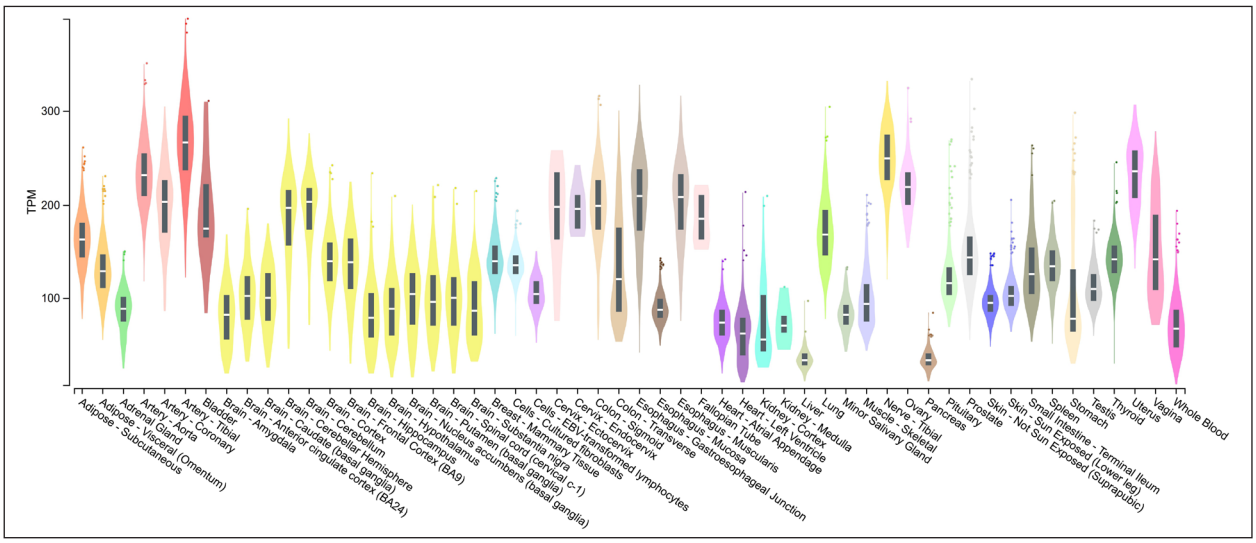

Figure 1. Transcriptional activity of the *ADD1* gene (rs4961) in organs and tissues.

tissue, and slightly less in the heart, kidneys, liver, pancreas, skeletal muscles (Figure 1).

We performed eQTL mapping on both sides of the transcription start site of the *ADD1* gene (rs4961) within ±10 Mb with further analysis of cis-acting or trans-acting regulatory variants, which predict the interactions nature. Two hundred associations of the *ADD1* gene rs4961 polymorphism with the expression of 23 genes in various tissues and organs were established (coefficient of linkage disequilibrium of alleles R^2=0.8). The most statistically significant associations are shown in Table 2.

The effective T-allele of rs4961 causes transcriptional repression of the NOP14-AS1 gene, the strongest was fond in blood cells, macrophages and CD16 neutrophils (β=–0.72; p=1.720e-8 and β=–0.63; p=9.130e-7) and slightly less was found in the basal nucleus of the brain striopallidary system, fibroblasts, breast gland, adipose tissue, aorta, testicles, central and peripheral nervous systems (CNS, PNS) (β=–0.45–/–0.34/; p≤1.210e-5–4.060e-14), leg and coronary arteries, liver, esophagus, subcutaneous fat, iPSC stem cells, lung, brain, prostate, colon, natural killer cells and skin (β=–0.32–/–0.19/; p≤0.001–1.340e-15). A significant *ADD1* gene expression decrease was found in CD4+ blood T cells (β=–0.68; p=5.050e-5), slightly less in CD16 blood neutrophils (β=–0.345; p=0.0081); besides T-allele causes suppression of adipose tissue and blood cells (β=–0.29; p=0.0017 and β=–0.18; p=4.500e-7).

In addition, the T-allele (rs4961) reduces the transcriptional activity of the MSANTD1 gene in kidneys and thyroid gland (β=–0.36; p=0.025 and β=–0.33; p=1.220e-6), the LRPAP1 gene in adipose tissue (β=–0.21; p=0.029), GRK4 gene in CD4+, CD8+ blood cells (β=–0.315; p=0.027 and β=–0.16; p=0.0021), RNF4 genes in the CNS (β=–0.17; p=2.530e-5), TNIP2 in kidneys (β=–0.48; p=5.070e-4) and MFSD10 in CD8+ blood lymphocytes (β=–2.03; p=0.042).

Tissue-specific eQTL expression of the *ADD1* gene (rs4961; chr4:2906707 [hg19]; region +/– 10M) was detected in 37 tissues, with 23 phenotypic traits, 200 associations of quantitative expression trait loci, among them 200 were cis-variants (cis-QTL), 0 trans variants (trans-QTL). We also established 73 methylation quantitative trait loci (mQTL) rs4961 of the *ADD1* gene, 2 protein quantitative trait loci (pQTL), 34 splicing quantitative trait loci (sQTL), 14 histone modification quantitative trait loci (hQTL), 23 quantitative trait loci of transcript usage traits (tuQTL) and 4 loci of incorporated long non-coding regions of RNA (lncRNA) (Figure 2).

The association of the *ADD1* gene (rs4961) 1378G>T polymorphism with the EAH development in general population was analyzed by binary logistic regression method. A marginally reliable dependence was established for the additive model (2TT+GT), the minor T-allele carriers had low probability of EAH developing [OR=0.52; 95% CI: 0.26–1.00; p=0.05]. Concomitantly, the epidemiological analysis confirmed an almost twice increased EAH risk in GG genotype (rs4961) carriers of the *ADD1* gene (OR=1.92; 95%CI: 0.90–4.10; p=0.066).

Polymorphic variants of the *ADD1* gene (rs4961) in this study showed no associations with the risk of CKD development (Table 3).

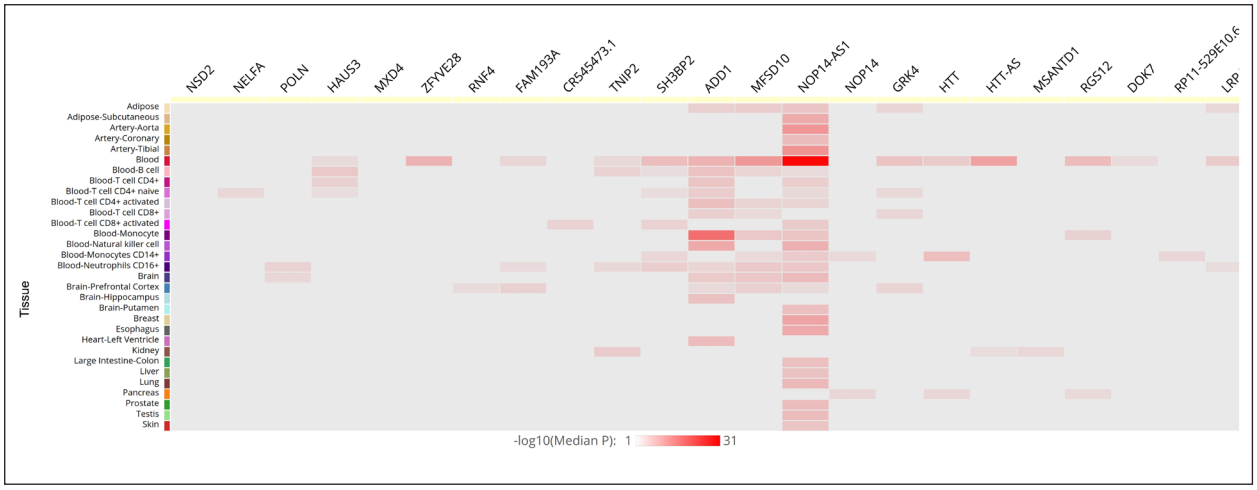

Figure 2. Heatmap of cis-regulation (plot+/-10M) of organ/tissue/cell dependent traits (vertical row) and molecular (genetic) eQTL traits (horizontal row) for rs4961 of the *ADD1* gene (chr4:2906707; hg19). Cell color saturation is the mean eQTL P-score. Abbreviations: eQTL – expression of the quantitative trait loci.

Table 2
Quantitative features of tissue-/cell-specific eQTL and genetic regulation for the alpha-adducin 1 gene (*ADD1*) polymorphism (rs4961) on chromosome 4

Gene expression changes	Tissue, cell, organ	HGVS effective interaction allele	β	S.E.	p-value
NOP14-AS1	Monocytes CD14	T	–0.7194	0.1276	1.720e-8
	Putamen (striopallidar system of the brain)	T	–0.4532	0.0977	1.210e-5
	CD16 neutrophils	T	–0.6302	0.1284	9.130e-7
	Breast	T	–0.4166	0.0702	1.280e-8
	Adipose tissue	T	–0.3727	0.0927	6.810e-5
	Fibroblasts	T	–0.3959	0.0493	4.060e-14
	Aorta	T	–0.3727	0.0464	1.880e-14
	Central nervous system	T	–0.3514	0.0462	1.630e-13
	Testicles	T	–0.3694	0.0780	3.530e-6
	Peripheral nervous system	T	–0.3397	0.0549	2.030e-9
	Liver	T	–0.3200	0.0756	2.900e-5
	Tibia Artery	T	–0.3236	0.0392	1.340e-15
	Esophagus	T	–0.3153	0.0439	3.300e-12
	Subcutaneous fat	T	–0.2990	0.0526	3.030e-8
	Coronary arteries	T	–0.3047	0.0633	3.200e-6
	iPSC stem cells	–	–0.2895	0.0883	0.0010
	Brain	T	–0.2700	0.0565	2.330e-6
	Lungs	T	–0.2750	0.0559	1.200e-6
	Prostate	T	–0.2700	0.0570	2.860e-6
	Natural blood killer cells	T	–0.2213	0.0413	1.400e-7
	Colon	T	–0.2451	0.0550	1.230e-5
	Skin	T	–0.1921	0.0474	5.730e-5
ADD1	Blood neutrophils CD16	–	–0.3452	0.1304	0.0081
	CD4+ blood T-cells	–	–0.6865	0.1624	5.050e-5
	Blood	T	–0.1803	0.0357	4.500e-7
	Adipose tissue	T	–0.2945	0.0933	0.0017
MSANTD1	Thyroid	T	–0.3264	0.0660	1.220e-6
	Kidneys	T	–0.3581	0.1568	0.0246
LRPAP1	Adipose tissue	T	–0.2060	0.0939	0.0287
GRK4	CD8+ blood T-cells	T	–0.1621	0.0523	0.0021
	CD4+ blood T-cells	T	–0.3155	0.1426	0.0269
MFSD10	CD8+ blood T-cells	T	–2.0302	0.9714	0.0423
TNIP2	Kidneys	–	–0.4763	0.1323	5.070e-4
RNF4	Central nervous system	T	–0.1684	0.0396	2.530e-5

Abbreviations: β – the effect's strength; eQTL – expression of the quantitative trait loci; *GRK4* – G protein-coupled receptor kinase 4 gene; HGVS – Human Genome Variation Society; iPSC – induced pluripotent stem cells; *LRPAP* – low-density lipoprotein receptor-related protein associated protein gene; *MFSD10* – major facilitator superfamily domain containing 10 gene; *MSANTD1* – Myb/SANT DNA binding domain containing 1 gene; *NOP14-AS1* – NOP14 antisense RNA 1 gene; *RNF4* – ring finger protein 4 gene; S.E. – standard error; *TNIP2* – TNFAIP3 interacting protein 2 gene.

All the examined individuals were divided into "sodium-sensitive" and "sodium-resistant" following the salt load reaction test. There were 54.0% (n=54) sodium-sensitive individuals among EAH patients, 81.48% (n=44) of them were women, 18.52% (n=10) were men. In the control group, there were 20.0% (n=12) sodium-sensitive subjects, 66.67% (n=8) of them were women and 33.33% (n=4) were men. The 46% of EAH patients (out of 100) were sodium-resistant, 76.09% (n=35) of them were women and 23.91% (n=11) were men. In the control group, entire 80.0% out of 60 individuals were sodium-resistant, 62.5% (n=30) of them were women and 37.5% (n=18) were men. The relative number of sodium-sensitive EAH patients prevailed over the sodium-sensitive ones in the control group; on the contrary, sodium-resistant subjects were less by 34.0% than in the control group (c^2=17.89; p<0.001). It should be noted that there were more sodium-sensitive hypertensive women by 14.81% compared to controls (c^2=12.47; p<0.001); and on the contrary, there were less sodium-sensitive hypertensive men by 14.81% (c^2=4.24; p=0.029) compare to controls (Figure 3).

The relative frequency of T-allele of the *ADD1* gene (1378G>T; rs4961) was higher by 11.9% in the sodium-sensitive EAH patients compared to controls (Figure 4). The number of sodium-resistant patients in the control group was higher by 22.2% (c^2=10.48; p=0.001). The frequency of severe EAH course (BP over ≥160/100 mm Hg) prevails among sodium-sensitive patients by 18.04% (46.3% vs. 28.3%; c^2=3.43; p=0.049).

The epidemiological analysis confirmed a 12-fold increased risk of sodium sensitivity in T-allele carriers of the *ADD1* gene (rs4961) in the EAH patients (OR 95%CI: 2.24–64.29; p=0.001), 4.71 times in women (OR 95%CI: 1.92–11.56; p<0.001) and 4.09 times in men (OR 95%CI: 1.03–16.28; p=0.041) (Table 4). Moreover, the sodium sensitivity presence increases the probability of EAH severe course (BP elevation ≥160/100 mm Hg) more than twice (OR=2.19; OR 95%CI: 1.00–5.05; p=0.049).

Discussion

Renal tubular function defect in hypertensive patients is the results of constitutive intracellular abnormalities in sodium transportation and liquid's volume, suggested a protein alteration in the actin cytoskeleton (Bianchi et al. 2005). A subtle protein of membrane skeleton, adducin, was identified in animal model of Milan hypertensive rat strain. Adducin is a heterodimeric cytoskeletal protein composed of α-subunit (Mr 103 kDa), β-subunit (Mr 97 kDa) and γ-subunit (Mr 90 kDa). These subunits are encoded by three genes (*ADD1*, *ADD2*, and *ADD3* in humans) located on different chromosomes (Matsuoka et al. 2000). Adducin facilitates the spectrin-actin lattice organization by promoting spectrin-actin binding. This protein is functioning in the presence of calcium and calmodulin and is phosphorylated by protein kinases A and C, tyrosine, and ρ-kinases. In addition, adducin is involved in signaling pathways, cell-to-cell contact transduction, and cell migration. Mutation of the *Add1* gene in the rat model accounts for the 50% of BP differences.

Many studies have examined the association between renal and cardiovascular diseases and the

Table 3
Polymorphic variants of the *ADD1* gene (rs4961) as predictors of chronic kidney disease in hypertensive patients

Predictor	*GG*-genotype of *ADD1* gene			*T*-allele of *ADD1 gene*		
	OR	OR 95%CI	p-value	OR	OR 95%CI	p-value
GFRcr ≤60 ml/min/1.73 m^2	0.62	0.22–1.76	0.261	1.62	0.57–4.58	0.365
GFRcys ≤60 ml/min/1.73 m^2	1.20	0.44–3.28	0.718	0.83	0.30–2.28	0.461

Abbreviations: GFRcr, GFRcys – glomerular filtration rate estimated after creatinine (cr), Cystatine-C (cys) blood values; OR – odds ratio; 95%CI – 95% confidential intervals.

Table 4
Polymorphic variants of the ADD1 gene (rs4961) as predictors of sodium sensitivity/sodium resistance in hypertensive patients

Predictors		Sodium Sensitivity			Sodium Resistance		
		OR	OR 95%CI	p-value	OR	OR 95% CI	p-value
ADD1 Genotypes	*GT–, TT–*	12.00	2.24–64.29	0.001	0.08	0.02–0.48	0.001
	GG–	2.40	0.91–6.34	0.074	0.42	0.16–1.10	0.061

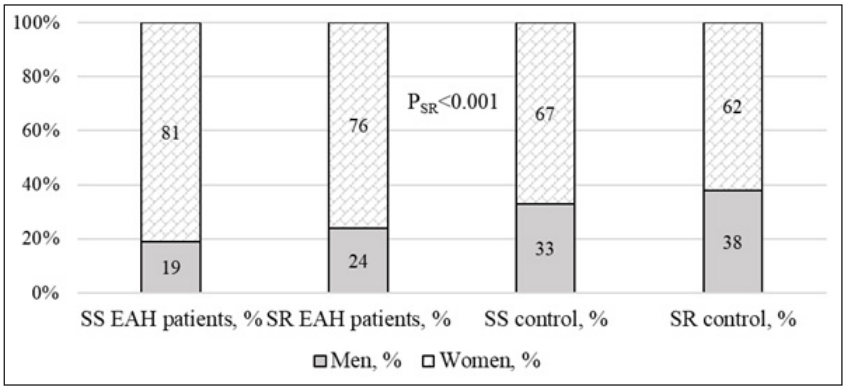

Figure 3. Observed distribution (%) depending on sodium sensitivity (SS) and sodium resistance (SR) and sex. Abbreviations: P_{SS}/P_{SR} – probability of differences with corresponding control group depending on sodium sensitivity (P_{SS})/sodium resistance (P_{SR}).

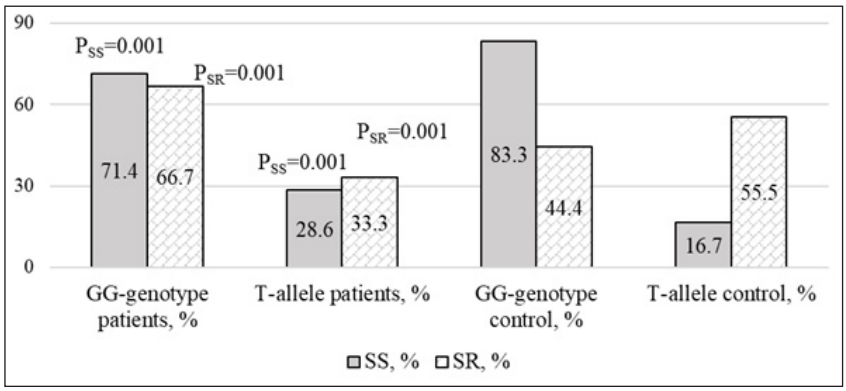

Figure 4. Observed subjects' distribution (%) depending on polymorphic variants of *ADD1* (1378G>T; rs4961) and sodium sensitivity (SS) and sodium resistance (SR) status. Abbreviations: P_{SS}/P_{SR} – probability of differences with corresponding polymorphic variant of *ADD1* gene (rs4961) of control group depending on sodium sensitivity (P_{SS})/sodium resistance (P_{SR}).

W (T-mutated) allele of *ADD1* gene. However, most of these studies were retrospective or had cross-sectional design, complicating the interpretation of their results. Some studies on cerebrovascular disease indicated an association between the *ADD1* W allele and hemorrhagic stroke, either alone or in combination with ischemic stroke (Chen et al. 2018; Psaty et al. 2002). However, no association with the *ADD1* W allele has been found when ischemic stroke was considered independently (Morrison et al. 2001). In our study the α-adducin gene (rs4961) did not demonstrate direct influence on hypertension appearance in observed population, or CKD developing. However, sodium sensitivity condition in mutated T (W)-allele carriers of the *ADD1* gene increases 12-fold the EAH risk in general (OR 95%CI: 2.24–64.29; p=0.001) regardless sex, 4.71 times in women (p<0.001) and 4.09 times in men (p=0.049). Moreover, the sodium sensitivity in EAH patients increases the likelihood of severe EAH course more than twice (OR=2.19; OR 95%CI: 1.00–5.05; p=0.049).

Some studies have demonstrated a linkage between coronary events and the *ADD1* W allele in hypertensive patients (Psaty et al. 2000; Morrison et al. 2002; Zhang et al. 2022). Others have reported a lower frequency of the W allele in survivors of myocardial infarction under 75 years of age compared to controls of the same age (Tobin et al. 2004). This finding might be due to the premature death of high-risk W allele carriers or contrary, indicating the protective effect of the W allele.

In the studies dedicated to polycystic kidney disease (autosomal dominant origin), the frequency of the *ADD1* W allele did not differ among small

subgroups (fewer than 100 patients) with varying durations of renal therapy and hemodialysis (Merta et al. 2003; Persu et al. 2003). Other researchers have reported an interaction between *ADD1* G460W and ACE I/D polymorphisms in the progression of renal failure (Narita et al. 2003), association with GFR reduction and effective renal plasma flow lowering in hypertensive patients on low-salt diet, or increasing in proteinuria (Beeks et al. 2004; Wang et al. 2004), and cancer development (Kiang and Leung 2004); in conjunction with *ADD3* gene mutation it might play a causal role in hypertensive-induced renal disease, cerebral vascular disorders or cognitive dysfunction, etc. (Gonzalez-Fernandez et al. 2022).

Among the potential mechanisms of sodium-resistant hypertension, changes in the metabolomic and lipidomic profile (insulin resistance, obesity, hormonal abnormalities), disturbances in signaling pathways including sodium and water transporters, endothelial dysfunction, elevated systemic vascular resistance, microbiome influence, genetic predisposition, etc. have been suggested (Ferguson et al. 2019; Chaudhary et al. 2021; Ishimwe et al. 2023; Sydorchuk et al. 2023b; Demirci et al. 2024; Katsuya et al. 2024; Lin et al. 2024). Apart from these mechanisms, there are some supportive co-factors of sodium sensitivity and EAH risk such as sex, age, race, baseline BP, physical activity, smoking, alcohol intake, potassium and sodium consumption, eGFR, immune disorders, and others (He et al. 2009; Ruan et al. 2021; Shi et al. 2022; Zhang et al. 2022; Zhang et al. 2023; Saleem et al. 2024). Our study confirms these findings, EAH individuals with sodium sensitivity were at an increased risk for EAH. However, further research is needed to understand the underlying mechanisms of sodium sensitivity and sodium resistance in EAH for improving hypertension management and prevention.

Previous multiple genetic studies have identified hundreds of genes associated with hypertension, as well as other cardio-vascular and renal diseases. Nonetheless, few of them were able to show some associations between the alpha-adducin-1 Gly460Trp polymorphism and salt-sensitive hypertension (Grant et al. 2002; Zhang et al. 2019, 2022), hypertensive-induced renal depletion with GFR reduction (Gonzalez-Fernandez et al. 2022), but none of them were done in Western Ukraine. Therefore, we endeavor to establish the *ADD1* gene rs4961 association with essential hypertension, sodium sensitivity and the functional kidneys state (after GFR) in Northern Bukovina region.

Conclusions

The expression of the eQTLs of *ADD1* gene (rs4961) (chr4:2906707 (hg19) was confirmed in 37 tissues and organs with 23 phenotypic traits. Two hundred associations of eQTL were revealed (200 cis-variants [cis-QTL], no trans-variants), additionally 73 methylation QTL (mQTL) rs4961, 34 splicing QTL (sQTL), 14 histone modification QTL (hQTL), 2 protein QTL (pQTL), 23 transcript utilization QTL (tuQTL) and 4 loci of incorporated long non-coding areas of RNA (lncRNA).

ADD1 gene (rs4961) polymorphic variants in this study are not associated with the probability of CKD developing in hypertensive patients. GG-genotype of the *ADD1* (rs4961) gene enhances the unreliable EAH risk almost twice (OR=1.92; 95%CI: 0.90–4.10; p=0.066).

Sodium sensitivity was observed in 41.25% (n=66) of subjects, 54.0% (n=54) of them were EAH patients and 20.0% (n=12) were controls (p<0.001). Sodium sensitivity in T-allele carriers of the *ADD1* gene (1378G>T; rs4961) increases 12-fold the EAH risk in population in general (p=0.001), 4.71 times in women (p<0.001) and 4.09 times in men (p=0.041). Sodium sensitivity in EAH patients elevates the likelihood of severe EAH course according to the BP elevation value more than twice (p=0.049).

Conflict of interest: *The authors declare no conflict of interest.*

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