

## AluYb8 insertion in the *WNK1* gene is not associated with hypertension in a Russian Caucasian population

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**ABSTRACT.** *WNK1* (With No-lysine Kinase 1), a serine-threonine kinase, regulates blood pressure by acting on various sodium transport-related ion channels. Several studies report a link between common variants of the *WNK1* gene and hypertension. No data exists on Russian populations. Our aim was to evaluate the association between the *WNK1* AluYb8 polymorphism and

hypertension susceptibility in a Russian Caucasian population. A total of 66 patients with arterial hypertension and 40 controls were screened by polymerase chain reaction. We evaluated the genotype distribution in the patient and control groups using a chi-square test and assessed the relationship between genotypes and hypertension. This preliminary study on a small number of individuals suggests the absence of any association between the AluYb8 polymorphism and increased blood pressure. The polymorphism therefore does not increase the risk of hypertension, and in our cohort, is not a major contributor to blood pressure variability. It is therefore not useful for evaluating predisposition to hypertension, at least in the Krasnoyarsk region.

**Key words:** *WNKI*; Arterial hypertension; Polymorphic AluYb8; Genetic Polymorphism; Blood pressure

## INTRODUCTION

Hypertension is a public health problem with high social and economic impact, affecting about 25% of the adult population. It is a major risk factor for the development of many frequent kidney and heart diseases (Mosterd et al. 1999; Kannel 2000; Griffin 2017).

Increased blood pressure is influenced by environmental factors (i.e. salt, alcohol consumption, obesity, physical activity, and chronic stress) and has a genetic component. Indeed, genetic studies on hypertension have identified various blood pressure regulating genes, belonging mainly to pathways involved in sodium homeostasis (Lifton et al. 2001; Vehaskari 2007), and various polymorphisms that influence blood pressure (Huang et al. 2008; Munroe et al. 2013).

*WNKI* (with No-lysine Kinase 1) is a serine/threonine protein kinase involved in the regulation of sodium and potassium transport in the distal convoluted tubules and cortical collecting ducts of nephrons, thereby contributing to modulation of blood pressure (Verissimo and Jordan 2001; Wilson et al. 2001). The human *WNKI* gene (29 exons) occupies ~160 kb on chromosome 12p13 and codes for multiple transcripts initiated by various promoters (Xu et al. 2000; Wilson et al. 2001; Delaloy et al. 2003).

Various mutations in the *WNKI* gene have been found to cause a rare familial hypertension and hyperkalemia syndrome (Gordon's syndrome) (Wilson et al. 2001). In addition, several common SNPs and haplotypes of *WNKI* have been associated with blood pressure and with the degree of hypertension (Tobin et al. 2005; Tobin et al. 2008; Newhouse et al. 2009; Cun et al. 2011; Putku et al. 2011). All these findings suggest that the *WNKI* gene may be a key regulator of blood pressure and that increased expression of *WNKI* may explain variability and susceptibility to hypertension.

In the present study, we investigated a polymorphic AluYb8 insertion in *WNKI* intron 10, previously considered a potential contributor to individual variability in blood pressure in different ethnic groups across Europe (Putku et al. 2011). Our final aim was to determine whether the association with hypertension susceptibility is maintained in a Russian Caucasian cohort. A study group and a control group were screened for the polymorphic sequence by PCR.

## MATERIAL AND METHODS

### Patients and healthy subjects

Sixty-six patients with hypertension and 40 control subjects were enrolled in the study conducted by the state educational institution of higher professional education, Krasnoyarsk State Medical University, in the largest cardiology clinic in the city of Krasnoyarsk, the Regional state budgetary health care institution Krasnoyarsk Inter district clinical hospital no. 20 I. S. Berzona in the period March 2014 to April 2017. Patients had to meet the following criteria to be enrolled in the study: 1) hypertensive syndrome (except secondary hypertension); 2)

age between 18 and 26 years; 3) Caucasian race; 4) written informed consent to participate in the study. Patients had to meet the following criteria: 24-hour blood pressure, systolic (day) >139 mmHg; dyslipidemia (total cholesterol >4.9 mmol/l, LDL cholesterol >3.0 mmol/l, triglycerides >1.7 mmol/l, HDL cholesterol <1.0 mmol/l); positive family history of early cardiovascular disease; adipopexia, body mass index  $\geq 30$  kg/m<sup>2</sup>; bicycle ergometry systolic blood pressure >200 mmHg. The control group was selected from members of the general population of Krasnoyarsk region (Siberia), without arterial hypertension.

### Genetic screening of *WNK1* intron 10 AluYb8

On the day of clinical evaluation, blood samples were collected in vials containing EDTA. DNA was extracted from 0.5 ml whole blood using an E.N.Z.A. blood DNA kit (Omega Bio-Tek; NORCROSS, GA, USA) according to the manufacturer's protocol. PCR amplification of the fragment encompassing the polymorphism was obtained by amplifying 100 ng DNA using AmpliTaq GOLD Fast PCR Master Mix (Applied Biosystems, USA) and 10 pmol of the forward and reverse primers 5'-GGGTAACCAACCCTTGAAGTAGG-3' and 5'-GGGTACTTCTCAAGTGATTAGGAGGA-3', respectively, according to the manufacturer's instructions. Reactions were conducted in a thermocycler (BIOER, Binjiang, China): 95°C for 10 min (initial denaturation), then 35 cycles at 95°C for 30 s (denaturation), 30°C for 30 s (annealing) and 72°C for 30 s (extension), followed by a final elongation at 72°C for 5 min. After amplification, PCR products were resolved by gel electrophoresis. The PCR fragment sizes were estimated in comparison with a 100-bp DNA ladder (Thermofisher). The expected wild type fragment had 353 bp while the polymorphic AluYb8 containing *WNK1* fragment contained 640 bp.

### Statistical analysis

We used the chi-square test to evaluate the distribution of polymorphic AluYb8 *WNK1* in patients and controls. Deviation from Hardy-Weinberg equilibrium was tested in the control group using an online tool (the Hardy-Weinberg 2-Allele Calculator <http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html>). The significance of the associations between the polymorphism and blood pressure was tested using logistic regression considering the genotype as dependent variable (Alu-dominant model, i.e. AA vs AB + BB) and age, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) as independent variables. Statistical analysis was conducted with MedCalc software (Mariakerke, Belgium). Statistical significance was set at P value <0.05.

## RESULTS

The general details of the 66 young adult males with arterial hypertension enrolled in this study are reported in Table 1.

**Table 1.** Clinical characteristics of the cohort of patients with arterial hypertension (N = 66) recruited in this study.

Characteristics	
Systolic blood pressure in a day (mmHg)	142.5 ± 12.9
Diastolic blood pressure in a day (mmHg)	83.1 ± 8.6
Body mass index (kg/m <sup>2</sup> )	26.23 ± 3.99
Mean Age (years)	23.20 ± 2.26

When our 40 sex-matched control subjects were screened by PCR to detect the polymorphism in intron 10 of *WNK1*, the polymorphic allele was detected in 12 (30%) and one (2.5%) subjects in heterozygous and homozygous state, respectively. The wild-type sequence was found in 67.5% of controls. The control group was found to be in Hardy-Weinberg equilibrium with respect to the polymorphism (P = 0.81).

The same analysis on our cohort of 66 hypertensive patients detected 17 (25.8%) and 2 (3%) subjects with the polymorphic allele in heterozygous and homozygous state, respectively, while the wild-type (WT) genotype was found in 71.2% of the cohort. Figure 1 shows a representative gel electrophoresis, illustrating the polymorphic profile of some DNA samples of our patient cohort. The frequency of the AluYb8 allele in the whole cohort was 16.5%. Genotype frequencies of the *WNK1* variant did not differ between patients and controls (P = 0.84).

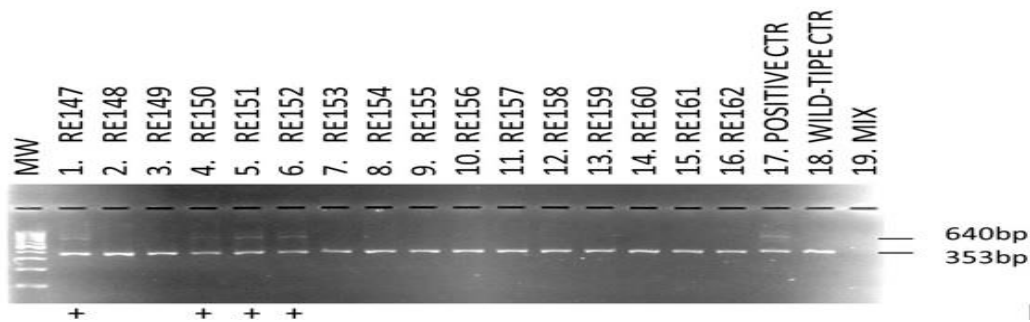
We compared various clinical parameters of the 66 patients in order to determine whether the presence or absence of the polymorphic AluYb8 allele in *WNKI* could be associated with differences in mean systolic or diastolic blood pressure over the period of a day (Table 2).

**Table 2.** Mean diastolic and systolic blood pressure values in a day in patients divided by genotype.

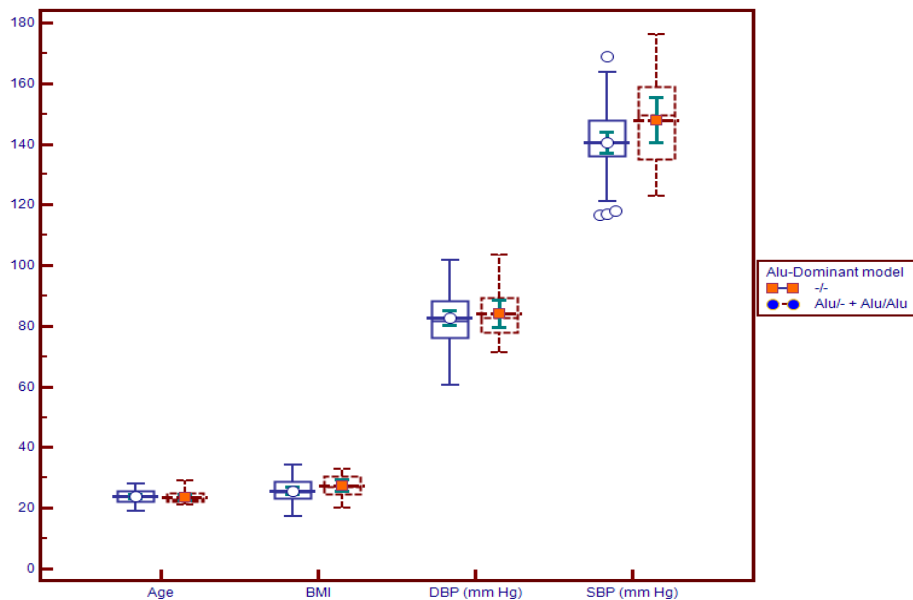
Genotype	N	Blood pressure in a day (mmHg)	
		Mean systolic blood pressure	Mean diastolic blood pressure
-/-	47	140.4	82.7
ALU/-	17	148.2	83.4
ALU/ALU	2	144.8	89.9

**Abbreviations:** - wild type sequence; ALU = Presence of the AluYb8 polymorphism in the *WNKI* gene.

The logistic regression statistics (Alu-dominant model) revealed that the *WNKI* AluYb8 insertion is not associated with blood pressure ( $P = 0.0880$ ) (Figure 2).



**Figure 1.** Representative post electrophoresis gel of samples amplified for the AluYb8 polymorphism in the intron 10 of the *WNKI* gene. Lane 2,3,7-16 shows a single amplified fragment of 353 bp representing a wildtype genotype, while lane 1,4-6 show two amplified fragments delineating the profile of a heterozygous DNA samples; in lane 17 there is the profile of a positive control (CTR), while on line 18 a *wild-type* control. Abbreviations: MW, 100-bp DNA ladder (Thermofisher); + DNA showing the AluYb8 polymorphism.



**Figure 2.** Box-plot diagram of variables distribution clustered according to the Alu-Dominant model: *wild-type* (-/-), *WNKI* AluYb8 heterozygous patients (Alu/-) + homozygous patients (Alu/Alu). Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

## DISCUSSION

Hypertension is a major public health problem, affecting one in four adults. It is a complex disease, influenced by environmental factors and genetics. Given its high social and economic impact, the need to define genetic features that may predispose to hypertension is strongly felt. Insights into hypertension have been gleaned through the study of rare monogenic hypertension syndromes and of mouse models (Shekarabi et al. 2017). Various blood pressure regulating genes, belonging mainly to the WNK-SPAK-cation-Cl<sup>-</sup> cotransporter pathways, have been identified. Genes involved in this cascade regulate sodium homeostasis through ion channels in the kidneys and other epithelia. Common variants in these genes are reported to contribute to blood pressure variations in the general population (Tobin et al. 2008; Newhouse et al. 2009; Persu et al. 2016).

In this study we investigated a polymorphic AluYb8 insertion in *WNK1* intron 10, hitherto considered a potential contributor to individual variability in blood pressure in different ethnic groups across Europe. In our cohort we found an AluYb8 allele frequency of 16.5%, which is in the range of frequencies observed in 18 different populations from Europe, Asia, and Africa (Putku et al. 2011). The latter authors reported a frequency of *WNK1* AluYb8 insertion ranging from 4.8% in Sub-Saharan Africans to 17% in Tatars (Putku et al. 2011). They also looked for an association between the polymorphic AluYb8 *WNK1* sequence and susceptibility to arterial hypertension in three different study groups (HYPEST-Estonians, CADCZ-Czech and BRIGHT-British study groups) and in a joint meta-analysis gathering about 3500 patients from the three study cohorts. The authors identified a statistically significant association between blood pressure (either systolic or diastolic) and presence of the AluYb8 polymorphism in the group as a whole. However, when they analysed the populations independently, significance was found in 2/3 cohorts for systolic blood pressure (HYPEST and BRIGHT), and in 1/3 cohorts for diastolic blood pressure (HYPEST). This suggests that ethnicity could influence the association between blood pressure and Alu insertion in *WNK1*. The same authors noted that the association was gender-specific and was only maintained among women in the joint meta-analysis and for both systolic and diastolic blood pressure in the HYPEST group. These findings are consistent with our preliminary results from this relatively small cohort of patients originating from Krasnoyarsk in Siberia, showing that the polymorphic AluYb8 insertion in *WNK1* intron 10 was not associated with arterial hypertension in our male Russian Caucasian population. Failure to replicate the association between blood pressure and polymorphism in this population could be due to genetic heterogeneity across populations, small effect sizes or low power. In future research, we plan to increase cohort size and to enrol both male and female Russian patients. This should enable us to verify whether the findings of Putku and co-workers are also confirmed in the Siberian cohort.

## CONCLUSION

In conclusion, our results indicate that the polymorphism does not increase the risk of hypertension, and were not a major contributor to blood pressure variability in our cohort of Russian males. This means that polymorphic sequence screening is not suitable for evaluating predisposition to hypertension in the Krasnoyarsk region.

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