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Association between *ATP2B1* and *CACNB2* polymorphisms and high blood pressure in a population of Lithuanian children and adolescents: a cross-sectional study

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ABSTRACT

Objectives Recently, genome-wide associated studies have identified several genetic loci that are associated with elevated blood pressure and could play a critical role in intracellular calcium homeostasis. The aim of this study was to assess the associations of *ATP2B1* rs2681472 and *CACNB2* rs12258967 gene polymorphisms with high blood pressure (HBP) among Lithuanian children and adolescents aged 12–15 years.

Study design and participants This was a cross-sectional study of a randomly selected sample of 646 12–15-year-old adolescents who participated in the survey 'The Prevalence and Risk Factors of HBP in 12–15 Year-Old Lithuanian Children and Adolescents (from November 2010 to April 2012)'. Anthropometric parameters and BP were measured. The participants with HBP were screened on two separate occasions. Subjects were genotyped *ATP2B1* rs2681472 and *CACNB2* rs12258967 gene polymorphisms using real-time PCR method.

Results The prevalence of HBP was 36.7%, significantly higher for boys than for girls. In the multivariate analysis, after adjustment for body mass index and waist circumference, boys with *CACNB2* CG genotype, *CACNB2* GG genotype and *CACNB2* CG +GG genotype had higher odds of having HBP in codominant (adjusted OR (aOR)=1.92; 95% CI 1.16 to 3.18, p=0.011; and aOR=2.64; 95% CI 1.19 to 5.90, p=0.018) and in dominant (aOR=2.05; 95% CI 1.27 to 3.30, p=0.003) inheritance models. Girls carrying *CACNB2* CG genotype and *CACNB2* CG +GG genotype had increased odds of HBP in codominant (aOR=1.82; 95% CI 1.02 to 3.24, p=0.044) and in dominant (aOR=1.89; 95% CI 1.09 to 3.28, p=0.023) inheritance models. Furthermore, significant associations were found in additive models separately for boys (aOR=1.72; 95% CI 1.20 to 2.46, p=0.003) and girls (aOR=1.52; 95% CI 1.05 to 2.20, p=0.027). No significant association was found between *ATP2B1* gene polymorphism and the odds of HBP.

Conclusions Our results indicate that *CACNB2* gene polymorphism was significantly associated with higher odds of HBP in Lithuanian adolescents aged 12–15 years.

Strengths and limitations of this study

- ► To our knowledge, this is the first study to determine the association of the *ATP2B1* rs2681472 polymorphism with high blood pressure (HBP) in adolescents aged 12–15 years in the Baltic states.
- ► Multivariate logistic regression analyses were conducted separately for boys and girls to evaluate the associations between *ATP2B1* rs2681472 and *CACNB2* rs12258697 gene polymorphisms and HBP under different inheritance models.
- ► As our study was a cross-sectional study in its design, we cannot determine a cause–effect relationship.

INTRODUCTION

Hypertension is one of the major risk factors of cardiovascular disease, and does not only affect middle-aged and elderly people, but is also increasing in prevalence among children and adolescents worldwide.[1 2] A well-known fact is that overweight and obesity, like family history of hypertension and low socioeconomic status, are related to elevated blood pressure (BP) in childhood, which is an important predictor of hypertension in adult life.[3 4] According to various epidemiological studies, the prevalence of elevated BP in children and adolescents ranges from 0.8% up to 5%.[5–7] Studies conducted in Lithuania have shown a higher prevalence of an increased BP among 3–7-year-old children (21.4%)[8] and a higher prevalence of prehypertension and hypertension among 12–15-year-old adolescents (12.6% and 22.5%, respectively).[9] The mortality rate from cardiovascular diseases in Lithuania is one of the highest in Europe.[10] Hypertension is a highly heritable trait, and around 40%–60% of individual differences in BP have a genetic basis.[11] Therefore, early identification of not only potential

environmental but also genetic risk factors for the development of hypertension is essential, as they are important diagnostic and prognostic molecular markers.

Genome-wide associated studies have identified several genetic loci that are associated with elevated systolic (SBP) or diastolic blood pressure (DBP),[12–14] but the mechanism by which a certain gene variant influences the risk of hypertension remains unclear. In our study, we selected genes (*ATP2B1* and *CACNB2*) that play a critical role in intracellular calcium homeostasis[15] and could be also involved in the pathogenic processes of hypertension. The *ATP2B1* gene encodes the plasma membrane calcium ATPase isoform 1, which removes bivalent calcium ions from eucaryotic cells against very large concentration gradients[16] and is responsible for controlling the contraction and dilation of vascular smooth muscles.[17] The *CACNB2* gene encodes the beta-2 subunit of a voltage-gated calcium channel and is a member of high voltage-gated calcium channel genes. The beta-2 subunit could interact with a pore-forming subunit of the calcium channel and could modulate calcium channel activity and blood pressure.[18]

The aim of our study was to investigate the associations of *ATP2B1* rs2681472 and *CACNB2* rs12258967 gene polymorphisms with HBP among Lithuanian children and adolescents aged 12–15 years.

METHODS

Study design and population

Blood pressure and anthropometric measurements performed in this study have been described in our previous publication.[19]

This was a cross-sectional study of children and adolescents who had participated in the baseline survey 'The Prevalence and Risk Factors of HBP in 12–15 Year-Old Lithuanian Children and Adolescents (Study 1, 2010–2012)' in Kaunas City and Kaunas District, which are the second-largest city and district in Lithuania.[19] The baseline survey that was based on a two-stage cluster sampling design enrolled 7638 children and adolescents aged 12 to 15 years who at the time of the examination (from November 2010 to April 2012) attended gymnasiums or secondary schools of all classes (grades 6, 7, 8 and 9). Data on clinically verified health disorders were collected from the subjects' medical records (Form No. 027–1/a). The present study examined data of a randomly selected sample of 646 participants (313 boys and 333 girls) aged 12–15 years who underwent BP and anthropometric examinations as well as a genetic examination of the saliva.

Measurements

Blood pressure measurement

BP was measured in the morning hours by a physician who was not wearing a white coat in a quiet environment. The adolescents were advised to avoid coffee, tea, energy drinks and physical exercises until the measurements were taken. Before the BP measurement, the participants were asked to sit still for 10 min. BP measurements were performed with an automatic BP monitor (Omron M6; Omron Halthcare Co., Kyoto, Japan) using a cuff of the appropriate size. BP was measured three times with a 5 min rest interval between the measurements, with the participant being in a sitting position. The average of three BP measurements was calculated and used in the analysis. According to 'The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents', normal BP (NBP) was defined as SBP and DBP less than the 90th percentile for sex, age and height, and HBP was defined as SBP and DBP ≥90th percentile for sex, age and height.[20] Prehypertension was defined as SBP or DBP levels between the 90th and the 95th percentile for sex, age and height, or if BP levels were ≥120/80 mm Hg even if below the 90th percentile.[20] All subjects with high BP (BP was in the ≥90th percentile) during the first screening underwent a second evaluation of BP measurements within 2–3 weeks.

Anthropometric measurement

All participants underwent anthropometric measurements while being barefoot and wearing only light clothes. Body weight and height of the participants were measured to the nearest 0.1 kg and 0.1 cm, respectively, by using a balance beam scale (Seca) and a portable stadiometer. The participants were grouped into three categories: normal weight, overweight, and obese, using the cut-off points of body mass index (BMI) by age and sex for children and adolescents proposed by the International Obesity Task Force (IOTF).[21]

Waist circumference (WC) was measured to the nearest 0.5 cm by using a flexible measuring tape (Seca) at a level midway between the lower rib margin and the iliac crest. Using the cut-off values of the percentiles of the WC according to the criteria of the National Health and Nutrition Examination Survey III,[22] the participants were grouped into the categories on the basis of their WC: below the 75th percentile (normal waist value), 75th<90th percentile and ≥90th percentile. High waist value was defined as WC ≥75th percentile.

Both BP and anthropometric measurements were performed at the subjects' schools by the same team of trained research personnel (physicians and research assistants).

Genetic analysis

Saliva samples were collected into tubes from each individual for DNA extraction. Genomic DNA was extracted using a commercial DNA isolation kit—the 'Genomic DNA Purification Kit' (Thermo Fisher Scientific, Lithuania) according to the manufacturer's instructions.

ATP2B1 rs2681472 and *CACNB2* rs12258967 gene polymorphisms in the subjects were genotyped using the real-time PCR technique with TaqMan allelic discrimination Assay-By-Design genotyping assays C_30872739_10 and C_31302374_10, according to the manufacturer's

Table 1 Demographic, anthropometric and BP characteristics of the study subjects by sex

Variables	Total (n=646)	Boys (n=313)	Girls (n=333)	P values*
Age (years)	13.27 (1.14)	13.31 (1.16)	13.24 (1.13)	0.607
Weight (kg)	52.50 (12.19)	54.03 (13.33)	51.07 (10.85)	0.015
Height (cm)	162.86 (9.45)	164.42 (10.51)	161.40 (8.09)	<0.001
BMI (kg/m^2)	19.61 (3.35)	19.76 (3.49)	19.46 (3.22)	0.429
WC (cm)	67.41 (8.66)	69.63 (8.93)	65.33 (7.87)	<0.001
SBP (mm Hg)	119.97 (14.75)	123.20 (16.13)	116.92 (12.61)	<0.001
DBP (mm Hg)	64.99 (8.01)	64.34 (7.99)	65.61 (7.99)	0.043

Values are mean±SD.
*Boys versus girls (t-test).
BP, blood pressure; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.

instructions (Applied Biosystems, Foster City, California, USA). Allele-specific fluorescence was analysed on the ABI 7900HT Sequence Detection System with SDS V.2.1 (Applied Biosystems).

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS V.20 for Windows. Categorical variables were expressed as numbers and percentage. The z-test was used to compare differences between the groups. Continuous variables were presented as mean values±SD. The normality of the distribution of the continuous variables was assessed by applying the Kolmogorov-Smirnov test. Comparisons between the groups were performed by applying the χ^2 test, Student's t-test and analysis of variance with Bonferroni post hoc test. The z-test was used to compare the proportions. The χ^2 test was used for the assessment of the Hardy-Weinberg equilibrium (HWE) for the distribution of genotypes. Logistic regression analyses were used to test for the associations of *ATP2B1* (rs2681472) and *CACNB2* (rs12258967) polymorphisms with HBP under five inheritance models based on the Akaike information criterion (AIC) to calculate the OR (95% CI) for *CACNB2* and *ATP2B1* polymorphism. ORs were adjusted for BMI and WC. The data were analysed separately for boys and girls. P values <0.05 were considered statistically significant.

Patient and public involvement

The schoolchildren and their parents or guardians were not involved in the design and conduct of the study.

RESULTS

The characteristics of the study population are given in table 1. Among 646 study subjects aged 12–15 years (mean±SD age: 13.27±1.14 years), 48.5% (n=313) were boys, and 51.5% (n=333) were girls. Boys were significantly heavier and taller, and had a significantly greater mean WC. They had significantly higher mean SBP and significantly lower mean DBP, compared with girls. There was no significant difference in mean age and BMI between these groups.

The characteristics of the study participants according to BP levels are presented in table 2. The overall prevalence of HBP was 36.7%. HBP was found more frequently in boys than in girls (45.7% vs 28.2%; p<0.001). Subjects aged 14 to 15 years were more likely to have HBP than subjects aged 12 to 13 years, but a significant difference was found only between the boys (56.3% vs 38.5%, p<0.05) (data not shown). The subjects with HBP had significantly higher mean values of weight, height, BMI and WC, compared with subjects with NBP. The prevalence of overweight, obesity and large WC (≥75th percentile) were much more common in participants with HBP than in the NBP group.

The distribution of the analysed genotypes was in the HWE (p>0.05) (table 3). No significant differences in the frequencies of *ATP2B1* and *CACNB2* genotypes or alleles between boys and girls were found.

The characteristics of the study subjects according to the *ATP2B1* genotypes are presented in table 4. Mean values of SBP and DBP were similar in groups with different genotypes separately for both sexes. Statistically significant differences between *ATP2B1* genotypes with respect to the mean values of age, weight, height and WC were observed only among boys. The subjects with *ATP2B1* AA genotype had higher mean values of anthropometric variables and SBP than *ATP2B1* AG and GG genotype carriers.

The characteristics of the study subjects according to *CACNB2* genotypes are presented in table 5. Mean values of weight, height, BMI, WC and DBP were similar in groups with different genotypes for both sexes separately, except for age and SBP among the boys. Furthermore, boys with the *CACNB2* GG genotype had the highest level of SBP.

The mean SBP and DBP levels of the subjects according to genotypes are given in table 6. No significant differences were observed comparing the mean values of SBP and DBP between the *ATP2B1* genotypes (AG vs AA, GG

Table 2 Characteristics of the study population according to the BP level

Characteristics	HBP (n=237)	NBP (n=409)	P value
Age, years, mean (SD)	13.30 (1.12)	13.26 (1.16)	0.458
Weight, kg, mean (SD)	58.33 (12.37)	49.13 (10.75)	<0.001
Height, cm, mean (SD)	165.18 (8.77)	161.52 (9.58)	<0.001
BMI, kg/m^2, mean (SD)	21.24 (3.54)	18.66 (2.84)	<0.001
WC, cm, mean (SD)	72.33 (9.15)	64.56 (6.93)	<0.001
SBP, mm Hg, mean (SD)	135.90 (9.84)	110.73 (7.40)	<0.001
DBP, mm Hg, mean (SD)	69.76 (8.04)	62.24 (6.58)	<0.001
Sex, n (%)			
Boys	143 (45.7)*	170 (54.3)	<0.001
Girls	94 (28.2)*	239 (71.8)	
Age, years, n (%)			
12–13	134 (34.6)	253 (65.4)	0.212
14–15	103 (39.8)	156 (60.2)	
BMI categories, n (%)			
Normal weight	172 (31.6)*	372 (68.4)	<0.001
Overweight	47 (58.8)*	33 (41.2)	
Obesity	18 (81.8)*	4 (18.2)	
WC percentile categories, n (%)			
<75th	176 (31.3)*	386 (68.7)	<0.001
75th-<90th	41 (67.2)*	20 (32.8)	
≥90th	20 (87.0)*	3 (13.0)	

The means were compared using t-test.
Categorical variables were compared using the χ^2 test.
*P<0.05 in comparison with NBP group (z-test).
BMI, body mass index; DBP, diastolic blood pressure; HBP, high blood pressure; NBP, normal blood pressure; SBP, systolic blood pressure; WC, waist circumference.

vs AA or GG vs AG), the *CACNB2* genotypes (CG vs CC, GG vs CC or GG vs CG) separately for both sexes within each BP group.

The boys who carried one or two copies of the *CACNB2* G allele were by 1.64 times more likely to have HBP than boys who had one or two copies of the *CACNB2* C allele (table 7).

The results of multivariate logistic regression analyses are presented in table 8. To examine the associations with HBP, we calculated adjusted ORs (aORs) adjusted by the BMI and WC for boys and girls separately. No significant association between *ATP2B1* genotypes and HBP was found either for boys or girls.

The results showed that boys who carried the *CACNB2* CG genotype, the *CACNB2* GG genotype and the *CACNB2* CG +GG genotypes had higher odds of having HBP than carriers of the *CACNB2* CC genotype did (in the codominant model: aOR=1.92; p=0.011 and aOR=2.64; p=0.018; in the dominant model: aOR=2.05; p=0.003, respectively). In girls, carriers of the *CACNB2* CG genotype and the *CACNB2* CG +GG genotypes had significantly higher odds of HBP than girls with the *CACNB2* CC genotype did (in the codominant model: aOR=1.82; p=0.044, and in dominant model: aOR=1.89; p=0.023). In addition, significant associations were found in additive models separately for both sexes.

DISCUSSION

Though hypertension in adolescents is less common than in adults, it has become a major concern in some countries over the past decade because of the evidence suggesting that HBP tracks from childhood to adulthood.[23] Children become hypertensive in adult years, particularly if they are obese as children or become obese as young adults.[3 4] In adults, primary hypertension is more common than secondary hypertension in the paediatric population.[24] In order to promote cardiovascular health in adults, it is strongly recommended to measure BP in all children ≥3 years of age.[25] It was examined that the model of prediction of adult hypertension consists of known physical and environmental childhood risk factors, family history of hypertension and novel genetic variants.[4] However, new findings of genome-wide association studies on adult hypertension are still little reflected in paediatric research. In the present study, we examined two newly identified genes together with measured hypertension

Table 3 Distribution of the *ATP2B1* and *CACNB2* genotypes and frequency of alleles in the study population

	HBP		NBP	
Characteristics	**Boys**	**Girls**	**Boys**	**Girls**
ATP2B1 genotypes, n (%)				
AA	106 (74.1)	73 (77.7)	121 (71.2)	166 (69.5)
AG	37 (25.9)	19 (20.2)	45 (26.4)	68 (28.4)
GG	0 (0)	2 (2.1)	4 (2.4)	5 (2.1)
ATP2B1 allele frequency				
A	0.87	0.88	0.84	0.84
G	0.13	0.12	0.16	0.16
CACNB2 genotypes, n (%)				
CC	56 (39.2)*	33 (35.1)	92 (54.2)	106 (44.4)
CG	67 (46.8)†	46 (48.9)	65 (38.2)‡	99 (41.4)
GG	20 (14.0)§	15 (16.0)	13 (7.6)¶	34 (14.2)
CACNB2 allele frequency				
C	0.63	0.60	0.73	0.65
G	0.37	0.40	0.27	0.35

*P<0.05 in comparison with NBP group (z-test).
†P<0.05 between *CACNB2* CC genotype and *CACNB2* CG genotype in boys with HBP (z-test).
‡P<0.05 between *CACNB2* CC genotype and *CACNB2* CG genotype in boys with NBP (z-test).
§P<0.05 between *CACNB2* CC genotype and *CACNB2* GG genotype in boys with HBP (z-test).
¶P<0.05 between *CACNB2* CC genotype and *CACNB2* GG genotype in boys with NBP (z-test).
ATP2B1, the plasma membrane calcium-transporting *ATPase 1* gene; *CACNB2*, beta-2 subunit of voltage-gated calcium channel; HBP, high blood pressure; NBP, normal blood pressure.

risk factors among Lithuanian children and adolescents aged 12–15 years.

The prevalence of hypertension in the paediatric population varies because BP relates to sex, age and height during childhood.[20 26 27] Nevertheless, many epidemiological studies reported the increasing prevalence of hypertension (from 11% to 22%) in children and adolescents between the ages of 3 and 18 years.[1 28–31] In our study, the prevalence of HBP was 36.7%, and was significantly higher in boys than in girls. Our results are consistent with those of other studies showing sex differences in BP.[32 33] Many studies have shown that obesity was associated with elevated BP.[23 34–36] In this study, we found that the prevalence of overweight, obesity and high WC (≥75th percentile) was much more common in participants with HBP than in the NBP group, which is in line with the findings of other researchers.

There is no doubt that the genetic element in the evaluation of hypertension is an important factor. However, the pathophysiological mechanisms of the association between gene polymorphisms and variations of BP are poorly understood. We examined *ATP2B1* rs2681472 and *CACNB2* rs12258967 variants located in an intron region of these genes. According to numerous Genome-wide association studies (GWAS) reports,[12–14] *ATP2B1* and *CACNB2* are involved in calcium transportation. In addition to well-known biological functions, calcium triggers muscle contraction and is the second messenger of hormones and growth factors.[37] A defect in the regulation of calcium and calcium signalling plays an important

Table 4 Characteristics of the population (means and SD) according to *ATP2B1* genotypes

	***ATP2B1* genotypes**			
Characteristics	**AA**	**AG**	**GG**	**P values**
Boys	n=227	n=82	n=4	
Age, years, mean (SD)	13.41 (1.14)	13.04 (1.20)*	12.75 (0.50)	**0.021**
Weight, kg, mean (SD)	55.40 (13.58)	50.41 (12.22)*	50.38 (5.19)	**0.012**
Height, cm, mean (SD)	165.47 (10.27)	161.63 (10.83)*	161.75 (7.93)	**0.015**
BMI, kg/m^2, mean (SD)	20.02 (3.64)	19.07 (3.02)	19.23 (1.15)	0.303
WC, cm, mean (SD)	70.54 (9.35)	67.40 (7.35)*	64.00 (1.41)	**0.012**
SBP, mm Hg, mean (SD)	123.90 (15.89)	121.89 (16.84)	110.58 (8.16)	0.218
DBP, mm Hg, mean (SD)	64.33 (7.95)	64.36 (8.36)	64.42 (3.34)	0.985
Girls	n=239	n=87	n=7	
Age, years, mean (SD)	13.22 (1.16)	13.31 (1.05)	13.14 (1.07)	0.751
Weight, kg, mean (SD)	51.64 (11.13)	49.92 (9.99)	46.00 (10.16)	0.205
Height, cm, mean (SD)	161.87 (7.83)	160.47 (8.74)	157.14 (7.34)	0.143
BMI, kg/m^2, mean (SD)	19.57 (3.39)	19.23 (2.75)	18.45 (2.75)	0.489
WC, cm, mean (SD)	65.69 (8.07)	64.67 (7.24)	61.29 (7.50)	0.152
SBP, mm Hg, mean (SD)	117.29 (12.65)	116.07 (12.82)	115.14 (8.93)	0.460
DBP, mm Hg, mean (SD)	65.86 (8.19)	65.20 (7.58)	62.52 (5.64)	0.472

*P<0.05 between *ATP2B1* AA genotype and *ATP2B1* AG genotype in boys (t-test).
ATP2B1, the plasma membrane calcium-transporting *ATPase 1* gene; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.

role in hypertension-associated vascular dysfunction.[38] The greatest amount of cellular calcium resides in the sarcoplasmic reticulum and mitochondria, resulting in its lower intracellular than extracellular concentration.[39] After depolarisation, calcium enters cardiomyocytes via L-type calcium channels. It activates ryanodine receptors on the sarcoplasmic reticulum to release calcium, and triggers muscle contraction. As the contraction ends, intracellular calcium returns to the sarcoplasmic reticulum via calcium ATPase or plasma membrane Ca^{2+} ATPase isoform 1 (PMCA1), which is encoded by *ATP2B1*.[40] The plasma membrane calcium/calmodulin-dependent ATPase is involved in calcium pumping from the cytosol to the extracellular compartment[41] and regulates the homeostasis of cellular calcium levels, which is important in controlling the contraction and dilatation of vascular smooth muscles.[42]

CACNB2 encodes the intracellular beta-2 subunit of a calcium channel (voltage-dependent L-type calcium channel), which is a member of the high voltage-gated calcium channel genes and serves as a target of calcium channel blockers.[43] These blockers, also known as calcium antagonists, act both on the cardiac tissue and on vascular smooth muscles. In cardiac tissues, a decrease in calcium levels directly results in a decrease of the force of the contraction of the heart, and thus a decrease in blood pressure. It was suggested that the beta-2 subunit could interact with the pore-forming alpha-1 subunit to modulate activity through conformational changes in the calcium channel. Mutations in genes could result in channel activation at more hyperpolarised membrane potentials, implicating an increased calcium influx in disease pathogenesis.[44] This may explain why mutations in *CACNB2* can influence intracellular calcium homeostasis and alter blood pressure.[18 45 46]

Studies have shown that *CACNB2* was associated with DBP,[12 13 23 47] with systolic pressure,[13 48–52] mean arterial pressure[53] and hypertension.[13 49–52] The Kaunas Cardiovascular Risk cohort study found that *CACNB2* CC carriers had the highest values of DBP in childhood. However, the logistic regression analysis failed to show significant associations between *CACNB2* (rs12258967) and the risk of hypertension.[23] *CACNB2* polymorphism (rs4373814) was found to be significantly associated with a decreased risk (OR=0.70, 95% CI 0.51 to 0.95) of hypertension in the Han Chinese population.[51] The association of the *CACNB2* polymorphism (rs11014166) with hypertension (OR=0.79, 95% CI 0.65 to 0.97) was also observed in a study of China.[52] A *CACNB2* intronic single-nucleotide polymorphism (SNP), rs1571787, had the most significant association with pulse pressure (minor allele frequency (MAF)=0.27, p=0.0003) in a large cohort study.[54] Our data showed that boys with the *CACNB2* GG genotype had the highest level of SBP. The dominant model of *CACNB2* adjusted for the BMI and WC showed that carriers of CG +GG genotypes had a significantly higher odds of HBP than carriers of the *CACNB2* CC genotype did (in boys and girls: aOR=2.05; p=0.003 and aOR=1.89; p=0.023, respectively).

Table 5 Characteristics of the population (means and SD) according to *CACNB2* genotypes

Characteristics	*CACNB2* genotypes			P values
	CC	CG	GG	
Boys	n=148	n=132	n=33	
Age, years, mean (SD)	13.11 (1.10)	13.45 (1.22)*	13.61 (1.09)†	**0.017**
Weight, kg, mean (SD)	54.10 (14.16)	53.27 (12.22)	56.75 (13.79)	0.405
Height, cm, mean (SD)	163.49 (10.88)	164.79 (9.68)	167.08 (11.71)	0.180
BMI, kg/m^2, mean (SD)	19.99 (3.69)	19.44 (3.33)	20.06 (3.11)	0.426
WC, cm, mean (SD)	69.98 (9.23)	68.72 (8.60)	71.73 (8.63)	0.172
SBP, mm Hg, mean (SD)	121.06 (16.13)	124.78 (16.27)*	126.52 (14.60)†	**0.037**
DBP, mm Hg, mean (SD)	64.06 (7.84)	64.43 (7.93)	65.25 (9.08)	0.730
Girls	n=139	n=145	n=49	
Age, years, mean (SD)	13.15 (1.16)	13.23 (1.07)	13.53 (1.16)‡	0.111
Weight, kg, mean (SD)	50.61 (11.42)	51.66 (10.89)	50.62 (8.99)	0.687
Height, cm, mean (SD)	161.33 (8.69)	161.88 (7.40)	160.20 (8.32)	0.455
BMI, kg/m^2, mean (SD)	19.30 (3.56)	19.56 (3.13)	19.60 (2.37)	0.239
WC, cm, mean (SD)	65.68 (8.71)	65.46 (7.60)	63.93 (5.81)	0.763
SBP, mm Hg, mean (SD)	115.63 (12.12)	118.40 (13.27)	116.23 (11.69)	0.225
DBP, mm Hg, mean (SD)	64.87 (8.03)	66.05 (7.94)	66.46 (8.01)	0.335

*P<0.05 between *CACNB2* CC genotype and *CACNB2* CG genotype in boys (t-test).
†P<0.05 between *CACNB2* CC genotype and *CACNB2* GG genotype in boys (t-test).
‡P<0.05 between *CACNB2* CC genotype and *CACNB2* GG genotype in girls (t-test).
BMI, body mass index; *CACNB2*, beta-2 subunit of voltage-gated calcium channel; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference

Table 6 Distribution of *ATP2B1* and *CACNB2* genotypes according to systolic and diastolic blood pressure in the study population

	HBP		NBP	
Characteristics	**SBP (mm Hg)**	**DBP (mm Hg)**	**SBP (mm Hg)**	**DBP (mm Hg)**
Boys				
ATP2B1 genotypes, mean (SD)				
AA	138.06 (10.10)	67.58 (7.51)	111.50 (7.36)	61.48 (7.22)
AG	137.48 (8.63)	68.14 (7.95)	109.07 (9.43)	61.24 (7.41)
GG	0 (0)	0 (0)	110.58 (8.16)	64.42 (3.34)
CACNB2 genotypes, mean (SD)				
CC	138.60 (9.82)	68.21 (7.11)	110.38 (7.57)	61.53 (7.20)
CG	138.15 (9.38)	67.57 (7.52)	111.00 (8.44)	61.19 (7.02)
GG	135.13 (10.57)	66.88 (9.36)	113.26 (8.78)	62.74 (8.38)
Girls				
ATP2B1 genotypes, mean (SD)				
AA	132.67 (8.03)	73.25 (7.62)	110.52 (7.20)	62.61 (6.05)
AG	134.35 (13.35)	72.05 (8.04)	110.97 (6.46)	63.28 (6.27)
GG	125.67 (0.00)	66.00 (10.37)	110.93 (6.48)	61.13 (3.51)
CACNB2 genotypes, mean (SD)				
CC	132.24 (9.60)	73.22 (7.24)	110.46 (7.16)	62.26 (6.33)
CG	134.17 (9.72)	72.07 (8.85)	111.07 (6.67)	63.25 (5.64)
GG	130.20 (6.58)	74.44 (4.63)	110.07 (7.26)	62.93 (6.51)

ATP2B1, the plasma membrane calcium-transporting *ATPase 1* gene; *CACNB2*, beta-2 subunit of voltage-gated calcium channel; DBP, diastolic blood pressure; HBP, high blood pressure; SBP, systolic blood pressure; NBP, normal blood pressure.

It remains unclear how *ATP2B1* could result in an increased risk of hypertension, but a significant association of a variant of the *ATP2B1* gene with blood pressure and hypertension was identified among Asian and European adults in GWAS.[12 13 55 56] The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium GWAS analysis showed that for rs2681472 in *ATP2B1,* the strongest signal for diastolic pressure – $p=3.7\times10^{-8}$ and with an OR for hypertension of 1.17 per risk allele.[13] A meta-analysis involving 15 909 cases and 18 529 controls confirmed that rs2681472 was significantly associated with the risk of hypertension in East Asians (OR=1.18, 95% CI 1.10 to 1.27, p<0.0001).[17] Another study among Chinese children confirmed a significant association of *ATP2B1* rs17249754 with the risk of hypertension (allelic OR=1.25, 95% CI 1.08 to 1.44, p=0.003).[57] However, five studies conducted in Korean,[58] Chinese[59] and Japanese populations[16 60 61] yielded inconsistent results. Our study found no significant association of *ATP2B1* with BP or with the risk of hypertension.

BP could be increased during puberty, which is related to rapid physical growth, the endocrine system

Table 7 Associations between *ATP2B1* and *CACNB2* polymorphisms and high blood pressure by sex (allelic models)

	Boys				Girls			
	HBP	NBP	OR		HBP	NBP	OR	
	N (%)	N (%)	(95% CI)	P value	N (%)	N (%)	(95% CI)	P value
ATP2B1 alleles								
A	249 (87.1)	287 (84.4)	1.00		165 (84.4)	400 (87.8)	1.00	
G	37 (12.9)	53 (15.6)	0.80 (0.50 to 1.30)	0.346	23 (15.6)	78 (12.2)	0.71 (0.42 to 1.21)	0.186
CACNB2 alleles								
C	179 (62.6)	249 (73.2)	1.00		112 (59.6)	311 (65.1)	1.00	
G	107 (37.4)	91 (26.8)	**1.64 (1.15 to 2.33)**	**0.004**	76 (40.4)	167 (34.9)	1.26 (0.88 to 1.81)	0.185

ATP2B1, the plasma membrane calcium-transporting *ATPase 1* gene; *CACNB2*, beta-2 subunit of voltage-gated calcium channel; HBP, high blood pressure; NBP, normal blood pressure.
ORs in bold text are statistically significant (p< 0.05).

and hormonal changes.[62] Epidemiological studies have demonstrated that adolescents' age was significantly associated with HBP.[63–65] A cross-sectional study in China including children and adolescents aged 5–18 years reported that subjects aged 12–14 and 15–18 years had a significantly increased risk of prehypertension, compared with those aged 5–8 years.[63] Data from the Healthy Kids Project showed that 12–17-year-old both boys and girls separately had a greater risk for prehypertension, hypertension and prehypertension/hypertension compared with 5–11-year-old participants.[64] Results from the 'Beijing children and adolescents (BP) study' showed that adolescents with hypertension during puberty had a higher risk of hypertension in adulthood than children with hypertension in prepuberty did.[65]

Elevated blood pressure at a young age predicts HBP in adulthood, which is the leading risk factor for cardiovascular diseases. Studies have shown that HBP in childhood correlates with carotid intima-media thickness, atherosclerosis, left ventricular hypertrophy and kidney failure in adulthood.[66–68] Childhood BP, overweight and obesity showed the largest OR for adult hypertension, followed by parental hypertension status in different longitudinal studies.[4 23 69] Screening children and adolescents for elevated blood pressure could identify hypertension at an early stage, decreasing the rate of progression of hypertension from childhood to adulthood and reducing the risk of cardiovascular disease in adulthood.[70]

Strengths and limitations

To our knowledge, this is the first study investigating the relationships between *ATP2B1* rs2681472 polymorphism and HBP among children and adolescents aged 12–15 years in the Baltic countries. Furthermore, we used multivariate logistic regression analyses to evaluate the associations under different inheritance models, separately for boys and girls. However, the present research has some limitations. The subject selection was limited to 12–15-year-old children and adolescents. Therefore, these results need to be confirmed in a large-scale study involving a larger population of younger children and older adolescents. In this study, BP readings were

Table 8 Associations between *ATP2B1* and *CACNB2* polymorphisms and high blood pressure by sex (multivariate analyses)

		Boys			Girls		
Models	Genotypes	aOR* (95% CI)	P value	AIC	aOR* (95% CI)	P value	AIC
CACNB2							
Codominant	CC	1.00			1.00		
	CG	**1.92 (1.16 to 3.18)**	**0.011**	412.01	**1.82 (1.02 to 3.24)**	**0.044**	367.15
	GG	**2.64 (1.19 to 5.90)**	**0.018**		2.15 (0.99 to 4.66)	0.054	
Dominant	CC	1.00			1.00		
	CG+GG	**2.05 (1.27 to 3.30)**	**0.003**	410.62	**1.89 (1.09 to 3.28)**	**0.023**	365.35
Recessive	CC+CG	1.00			1.00		
	GG	1.93 (0.90 to 4.14)	0.090	416.52	1.53 (0.77 to 3.05)	0.228	369.32
Overdominant	CC+GG	1.00			1.00		
	CG	1.59 (0.99 to 2.56)	0.055	415.74	1.44 (0.86 to 2.41)	0.162	368.76
Additive		**1.72 (1.20 to 2.46)**	**0.003**	410.37	**1.52 (1.05 to 2.20)**	**0.027**	365.78
ATP2B1							
Codominant	AA	1.00			1.00		
	AG	1.11 (0.66 to 1.89)	0.691	417.10	0.72 (0.39 to 1.34)	0.302	389.63
	GG				0.93 (0.16 to 5.59)	0.941	
Dominant	AA	1.00			1.00		
	AG+GG	1.02 (0.61 to 1.72)	0.932	419.43	0.74 (0.41 to 1.34)	0.318	369.70
Recessive	AA+AG	1.00			1.00		
	GG			415.26	1.01 (0.17 to 6.04)	0.992	370.72
Overdominant	AA+GG	1.00			1.00		
	AG	1.15 (0.68 to 1.95)	0.602	419.17	0.72 (0.39 to 1.34)	0.304	369.63
Additive		0.92 (0.57 to 1.50)	0.750	419.34	0.79 (0.46 to 1.34)	0.375	369.91

*Adjusted for body mass index and waist circumference.
AIC, Akaike information criterion; aOR, adjusted OR; *ATP2B1*, the plasma membrane calcium-transporting *ATPase 1* gene; *CACNB2*, beta-2 subunit of voltage-gated calcium channel.
ORs in bold text are statistically significant (p< 0.05).

obtained by an automatic oscillometric BP monitor, although, according to the Fourth Report of the National High Blood Pressure Education Program (NHBPEP), HBP readings should be repeated by using auscultation.[20] The present study did not evaluate pubertal status and biochemical parameters. In addition, there was no adjustment for family history of hypertension because information obtained from self-reports was lacking. The design of our study did not allow us to determine the cause–effect relationship.

CONCLUSION

In conclusion, *CACNB2* rs12258967 gene polymorphism was significantly associated with higher odds of HBP in Lithuanian adolescents aged 12–15 years. *ATP2B1* rs2681472 gene polymorphism was not associated with the odds of HBP. Further studies, particularly those evaluating the effect of genetic–anthropometric–environmental interactions on blood pressure, could help us to understand new pathophysiological mechanisms of the regulation of BP and to find potential targets for treatments.

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