Positive Association of the *JAG1* rs1327235 Genotype with Coronary Artery Disease in Men, the Tampere Adult Population Cardiovascular Risk Study

Tarja Kunnas¹, Seppo T Nikkari^{1*}

¹Department of Medical Biochemistry, Faculty of Medicine and Health Technology,
University of Tampere and Fimlab laboratories, Tampere, Finland

* Corresponding author:

Seppo T. Nikkari, MD, PhD, Department of Medical Biochemistry, Faculty of Medicine and Health Technology, FI-33014 Tampere University, Finland. E-mail: seppo.nikkari@tuni.fi

Running title: *JAG1* gene variant rs1327235 and coronary disease

Abstract

Aims. The intronic single nucleotide polymorphism (SNP) rs1327235 (A>G) close to the *JAG* gene has been implied to be involved in blood pressure physiology in a genome wide association study (GWAS). We wanted to study whether it associates with hypertension and coronary artery disease (CAD) in the Tampere adult population cardiovascular risk study (TAMRISK).

Materials and Methods. We analyzed a Finnish periodic health examination cohort of 191 men with diagnosed hypertension and 295 controls. Samples were genotyped for the *JAG1* rs1327235 polymorphism using Competitive Allelic Specific PCR (KASP). Incidence of CAD was followed up by self-report and the National Hospital Discharge Registry (HILMO)

Results. There was no association of *JAG1* rs1327235 genotypes with hypertension at the age of 50. When the subjects were followed up to the age of 60, those with genotype GG had higher prevalence of CAD (17.9%), compared to the A-allele (9.7%) ($p=0.036$). When prevalence of CAD was adjusted by BMI and total cholesterol, the OR for GG genotype was 2.19 ($p=0.029$, CI 1.084 – 4.429) compared to A-allele carriers. In addition, the GG genotype associated with higher total cholesterol and LDL-cholesterol values, compared to the A-allele.

Conclusions. Our findings suggest that the variations in *JAG1* rs1327235 may be involved in CAD and cholesterol metabolism.

Keywords: Genetic variation, *JAG1* gene, Coronary artery disease

Introduction

The Notch signaling pathway is a highly conserved signaling mechanism between ligands on cell membranes and receptors on adjacent cells. JAGGED1 (JAG1) is a ligand for NOTCH transmembrane receptors that is expressed by the *JAG1* gene. Binding of JAG1 to the NOTCH receptor results in cleavage of the receptor, and its intracellular domain translocates to the nucleus forming an active transcriptional complex that regulates transcription of target genes. Mutations in *JAG1* or *NOTCH2* are responsible for the Alagille syndrome, which affects the vasculature and other organs, including liver, heart, and kidney (Gilbert and Spinner. 2017) . There are embryonic lethality and vascular defects in mice lacking *JAG1* (Xue et al. 1999). Endothelial expression of JAG1 may be required for vascular smooth muscle development (High et al. 2008). A genome-wide association study (GWAS) of systolic and diastolic blood pressure has presented *JAG1* as a gene suspected to be involved in blood pressure physiology (International Consortium for Blood Pressure Genome-Wide Association Studies et al. 2011). The blood pressure-associated genetic locus *JAG1* rs1327235 was replicated among samples from East African subjects, where it associated with diastolic blood pressure (Kayima et al. 2017). Many of the newly identified variants affecting blood pressure are valid across ethnicities (Ehret and Caulfield. 2013). We wanted to study whether *JAG1* rs1327235 associates with hypertension and coronary artery disease (CAD) in the Tampere adult population cardiovascular risk study (TAMRISK).

Materials and Methods

Subjects

TAMRISK study data was collected from periodic health examinations (PHE) done for 50-year-old men and women living in Tampere, a city in southern Finland with 220 000

inhabitants (Maatta et al. 2015). A public health nurse did the PHE for the study subjects in 2003. Height (cm) and weight (kg) were recorded, and blood pressure measurement (mmHg) was done using a calibrated mercury sphygmomanometer. Serum total cholesterol, HDL-cholesterol, and triglycerides were determined after an overnight fast by standard techniques. The nurse conducted an interview using a structured questionnaire about health and health-related behavior. Current and previous diseases were identified based on self-report, including hypertension, which had been diagnosed by a physician using normal healthcare procedures. At that time, physicians diagnosed hypertension when blood pressure readings were consistently 140/90 mmHg or above. Separately of the physical examination, we collected during years 2006–2010 from participants by mail buccal swabs for DNA extraction and a permissions form to use PHE information and national registry data. Using the subject's national identity code, data on hospitalizations including ICD-10 codes for discharge diagnoses were obtained from the National Hospital Discharge Registry (HILMO) maintained by the National Institute of Health and Welfare. Prevalence of ischemic heart diseases (I20-I25) were followed up from 2005 to 2014 until the subjects were on the average 60 years old. In follow-up of the genotyped subjects, there were 54 men with coronary artery disease (CAD). All participants gave informed consent and the Ethics Committees of the Tampere University Hospital and the City of Tampere approved the study.

Cases (n=191) were subjects who had hypertension at the age of 50 years (as diagnosed by a physician) and for each case, at least one normotensive control subject (n=295) were chosen in order of admission from the PHE cohort (n=6000). The present study population at the age of 50 years thus included 486 men.

Genotyping

DNA was extracted from buccal swabs using a commercial kit (Qiagen Inc., Valencia, Calif., USA). Genotyping was performed using Competitive Allelic Specific Amplification (KASP) genotyping services at KBioscience Institute, UK. Details of this method can be obtained from <https://www.lgcgroup.com/genotyping/>.

Statistical analysis

Logistic regression, one-way ANOVA or T-test for continuous variables and Chi-square test or Fisher's exact test for categorical variables were applied for the comparison of cases, controls and genotype groups. Analyses were carried out using SPSS 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Clinical characteristics of the male group of 191 hypertensive subjects and 295 controls at the age of 50 years are shown in Table 1. Men with hypertension had higher BMI, serum triglycerides, systolic- and diastolic blood pressure compared to controls. There was no association of *JAG1* rs1327235 genotypes with hypertension. The *JAG1* rs1327235 genotype frequencies for the whole study population were AA 31.8%, AG 50.9%, and GG 17.3%. The genotypes were in Hardy-Weinberg equilibrium (Chi-square=0.159, $p>0.05$). Allele frequencies were A 57.2%, and G 42.8%.

When the subjects were followed up to the age of 60, those with genotype GG had higher prevalence of CAD (17.9%), compared to the A-allele (9.7%) ($p=0.036$) (Table 2). When prevalence of CAD was adjusted by BMI and total cholesterol determined at the age of 50, the OR for GG genotype was 2.19 ($p=0.029$, CI 1.084 – 4.429), compared to A-allele

carriers. At the age of 50 years, the GG genotype associated with higher total cholesterol and LDL-cholesterol values, compared to the A-allele.

Discussion

A GWAS of systolic and diastolic blood pressure has presented *JAG1* as a gene suspected to be involved in blood pressure physiology (International Consortium for Blood Pressure Genome-Wide Association Studies et al. 2011), since the *rs1327235* SNP (A>G) is located in an intron region, where the nearest gene is *JAG1* (Kayima et al. 2017). In fact, results from GWAS have identified a vast number of potentially functional intronic variants affecting disease (Cooper. 2010). However, it may also be possible that *rs1327235* is in linkage disequilibrium with a functional polymorphism. In contrast to earlier studies (International Consortium for Blood Pressure Genome-Wide Association Studies et al. 2011) (Kayima et al. 2017), we did not find an association of *rs1327235* with blood pressure. However, a limitation of our study is that it was restricted to a Finnish population, and the results may not necessarily be extrapolated to other ethnic populations.

The Alagille syndrome is caused by rare and abnormal mutations in *JAG1* or the receptor, *NOTCH2* (Gilbert and Spinner. 2017). It affects the vasculature among other organs (Gilbert and Spinner. 2017). Earlier studies have found abnormalities of the aorta, pulmonary, intracranial, renal, celiac, superior mesenteric, and subclavian arteries (Kamath et al. 2004). When the men in our study were followed up to the age of 60, those with minor genotype GG had higher prevalence of CAD compared to the A-allele. This difference remained even when prevalence of CAD was adjusted by BMI and total cholesterol, suggesting that *rs1327235* might be an independent risk factor for this disease.

In addition to systems manifestations of the Alagille syndrome, most of cases with *JAG1* mutations are associated with hypercholesterolemia due to liver function abnormalities (Liu et al. 2018) (Hannoush et al. 2017) . In line with this finding, the *JAG1* rs1327235 minor genotype GG associated with higher serum total cholesterol and LDL-cholesterol values, compared to A-allele carriers, in our male study population.

The *JAG1* polymorphism rs1327235 is a DNA sequence variation that is common in the population. When other *JAG1* polymorphisms were analyzed in a Mexican population, there was no statistical allele frequency difference identified between patients with Alagille syndrome and controls in 12 previously known polymorphisms, only with one exception, rs2273060 (Vazquez-Martinez et al. 2013). Alagille syndrome with loss of function mutations is at the other end of the spectrum, but it may be that common variations in *JAG1* have also potential to compromise health.

Acknowledgments

The expert technical assistance by Mirka Pietiläinen and Nina Peltonen is gratefully acknowledged. This study was supported by grants from Competitive research funding of the Pirkanmaa Hospital District.

Author Disclosure Statement

No competing financial interests exist.

References

- Cooper DN (2010) Functional intronic polymorphisms: Buried treasure awaiting discovery within our genes. *Hum Genomics* 4:284-288.
- Ehret GB, Caulfield MJ (2013) Genes for blood pressure: an opportunity to understand hypertension. *Eur Heart J* 34:951-961.
- Gilbert MA, Spinner NB (2017) Alagille syndrome: Genetics and Functional Models. *Curr Pathobiol Rep* 5:233-241.
- Hannoush ZC, Puerta H, Bauer MS, *et al* (2017) New JAG1 Mutation Causing Alagille Syndrome Presenting With Severe Hypercholesterolemia: Case Report With Emphasis on Genetics and Lipid Abnormalities. *J Clin Endocrinol Metab* 102:350-353.
- High FA, Lu MM, Pear WS, *et al* (2008) Endothelial expression of the Notch ligand Jagged1 is required for vascular smooth muscle development. *Proc Natl Acad Sci U S A* 105:1955-1959.
- International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, *et al* (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478:103-109.
- Kamath BM, Spinner NB, Emerick KM, *et al* (2004) Vascular anomalies in Alagille syndrome: a significant cause of morbidity and mortality. *Circulation* 109:1354-1358.
- Kayima J, Liang J, Natanzon Y, *et al* (2017) Association of genetic variation with blood pressure traits among East Africans. *Clin Genet* 92:487-494.
- Liu Y, Wang H, Dong C, *et al* (2018) Clinical Features and Genetic Analysis of Pediatric Patients with Alagille Syndrome Presenting Initially with Liver Function Abnormalities. *Curr Med Sci* 38:304-309.
- Maatta KM, Nikkari ST, Kunnas TA (2015) Genetic variant coding for iron regulatory protein HFE contributes to hypertension, the TAMRISK study. *Medicine (Baltimore)* 94:e464.
- Vazquez-Martinez ER, Varela-Fascinetto G, Garcia-Delgado C, *et al* (2013) Polymorphism analysis and new JAG1 gene mutations of Alagille syndrome in Mexican population. *Meta Gene* 2:32-40.
- Xue Y, Gao X, Lindsell CE, *et al* (1999) Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum Mol Genet* 8:723-730.

Table 1. Clinical characteristics of the male study population at the age of 50 years stratified according to hypertension.

	Hypertension (n=191)	Controls (n=295)	p-value*
Body mass index, kg/m ² (SD)	29.2 (4.9)	25.9 (3.5)	<0.001
Cholesterol, mmol/l (SD)	5.33 (1.00)	5.32 (0.95)	0.887
HDL cholesterol, mmol/l (SD)	1.41 (0.36)	1.50 (0.36)	0.091
Triglycerides, mmol/l (SD)	1.75 (1.21)	1.39 (0.90)	0.001
LDL cholesterol, mmol/l (SD)	3.16 (0.90)	3.21 (0.83)	0.548
Systolic blood pressure, mmHg (SD)	143.8 (16.8)	130.8 (14.7)	<0.001
Diastolic blood pressure, mmHg (SD)	94.0 (9.4)	86.0 (9.0)	<0.001
<i>JAG1</i> rs1327235 genotypes AA/AG/GG, %	31.4 / 51.8 / 16.8	32.0 / 50.3 / 17.7	0.942

SD, standard deviation. *T-test or chi-square test.

Table 2. Clinical characteristics (means $\pm$ SD) of the male study population at the age of 50 years stratified according to *JAGI* rs1327235 genotypes. Prevalence of coronary artery disease was followed up to the age of 60 years.

	AA (154)	AG (248)	GG (84)	P value * AA vs. AG vs. GG	P value * AA vs. (AG+GG)	P value * GG vs. (AA+AG)	P value ** GG vs. (AA+AG)
Coronary artery disease % (n)	11.7 (18)	8.5 (21)	17.9 (15)	0.059	0.759	0.036	0.029
Body mass index kg/m ² (SD)	27.0 (4.7)	27.4 (4.5)	26.9 (3.8)	0.605	0.515	0.599	
Total cholesterol, mmol/L (SD)	5.31 (0.91)	5.24 (0.91)	5.60 (1.18)	0.015	0.855	0.005	
HDL cholesterol, mmol/L (SD)	1.48 (0.36)	1.45 (0.36)	1.50 (0.38)	0.420	0.587	0.331	
Triglycerides, mmol/L (SD)	1.52 (1.09)	1.53 (1.04)	1.55 (1.04)	0.985	0.884	0.893	
LDL cholesterol, mmol/L (SD)	3.17 (0.82)	3.13 (0.82)	3.42 (1.01)	0.036	0.693	0.011	
Systolic blood pressure, mmHg (SD)	134.9 (18.1)	136.0 (15.7)	137.8 (17.2)	0.453	0.361	0.270	
Diastolic blood pressure, mmHg (SD)	88.2 (10.6)	89.5 (9.4)	89.7 (10.1)	0.337	0.142	0.558	

SD, standard deviation. * Chi-square test, Fisher's exact test, One-way ANOVA or T-test. ** Logistic regression adjusted by BMI and total cholesterol. P values <0.05 are in bold.