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Novel human genome variants associated with alcohol use disorders identified in a Lithuanian cohort

Karolis Baronas*,

Tautvydas Rančelis,

Aidas Pranculis,

Ingrida Domarkienė,

Laima Ambrozaitytė,

Vaidutis Kučinskas

Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Background. Alcohol use disorder (AUD) is a chronic relapsing brain disease characterized by compulsive alcohol use, loss of control over alcohol intake, and a negative emotional state when not using (1). Abusive alcohol consumption directly affects a person's physical and psychological health and social life. The World Health Organization has shown that Lithuania is a leading country in pure alcohol consumption in the world (2). The aim of this study is to find novel genome variants that are associated with the AUD in the Lithuanian cohort.

Materials and methods. A case-control study included 294 individuals of Lithuanian ethnicity, who were divided into two groups based on their habits of alcohol use. Single nucleotide polymorphism array analysis was performed using Illumina HiScanSQ™ genome analyzer.

Results. Our study showed that rs686141T>C variant in *NALCN* gene is more prevalent in the non-drinker group compared to the alcohol drinker group (relative allele frequency, respectively: 0.38 and 0.27, OR = 0.60 (CI 95% 0.37–0.98), $p = 0.0408$). Meanwhile, rs6354C>A, in *SLC6A4* gene, variant's genotype distribution showed statistically significant difference between the non-drinker and alcohol drinker group (distribution of genotypes in the case group: 9/72/172 (CC/CA/AA) and in the control group: 5/7/29, $p = 0.0264$).

Conclusion. We analyzed 23 genes associated with AUD and identified two novel genome variants (rs686141T>C and rs6354C>A). The study shows that genome analysis is an important tool for AUD research. The results supplement the known information about genes associated with AUD.

Keywords: Alcohol Use Disorder, Illumina HiScanSQ, genotyping, *NALCN*, *SLC6A4*

Correspondence to: Karolis Baronas, Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, 2 Santariškių St., Vilnius 08661, Lithuania. Email: karolis.baronas@mf.vu.lt

INTRODUCTION

Alcohol use disorder (AUD) is a chronic relapsing brain disease characterized by compulsive alcohol use, loss of control over alcohol intake, and a negative emotional state when not using (1). In 2010, alcohol-attributable cancer, liver cirrhosis, and injury caused 1,500,000 deaths or 2.8% of all deaths worldwide (3). According to the World Health Organization, Lithuania is a leading country in pure alcohol consumption in the world with 18.2 litres of pure alcohol consumption per capita within a calendar year (2).

Recent studies have shown strong evidence to support the hypothesis that AUD is a complex disease with hereditary and environmental effects, and with 50–60% heritability (4). Genes involved in vulnerability to AUD include genes that act on common metabolic pathways involved in addiction to different substances and predisposition to other psychiatric disorders. Alcohol-specific genes include genes for metabolic enzymes involved in the metabolism of ethanol, as well as genes encoding gatekeeper molecules such as receptors or neurotransmitters (5).

An understanding of the molecular mechanisms and metabolic pathways involved in excessive alcohol consumption is crucial for treatment of and screening for AUD. The aim of this study was to find novel genome variants associated with AUD in a Lithuanian cohort.

METHODS

Single nucleotide polymorphism array analysis was performed on 294 selected Lithuanians, whose family members were born in Lithuania for three generations, using Illumina HiScanSQ™ genome analyzer. The case groups (alcohol drinker group) and the control (non-drinker group) were formed based on questionnaire. Descriptive statistics of the study groups are shown in Table 1.

DNA was extracted from venous blood samples using either MagneSil® Genomic, Large Volume System (Promega Corp., USA) automated for the TECAN Freedom EVO® platform (TECAN Group Ltd., Switzerland), according to the manufacturer's instructions, or the phenol-chloroform extraction method. We used Illumina HiScanSQ™ platform and Illumina HumanOmniExpress-12 v1.1 array, adhering to the Infinium® HD Assay Ultra Protocol Guide (Illumina Inc., USA). The genotyping data visualization, primary quality control analysis, filtering, and output file generation were accomplished using the Illumina GenomeStudio v2011.1 Genotyping Module software.

Genotyping data contained 98 genome variants from 23 genes associated with AUD. Genome variants were picked for further investigation from these genes: *ADH1A, ADH1B, ADH1C, ADH4, ADH5, ADH6, ADH7, ALDH1A1, ALDH2, CYP2E1, CHRNA3, CHRNA5, GABRA2, OPRM1, HTR2A, HTR3B, NALCN, COMT, DPYSL2, GAD2, SLC6A4, ANKK1, NPY*. Genome variants, whose minor allele frequency was larger than 0.01, were selected for frequency evaluation in the Lithuanian population. The Hardy-Weinberg equilibrium and allele/genotype frequencies were determined using the PLINK v1.90b3.44 64-bit (2016-11-17) software (6). Pearson's chi-squared test and Fisher's exact test (for genotype counts less than 5) were used to evaluate the results.

RESULTS

Minor allele frequency (MAF) evaluation was done and only 65 genome variants with MAF greater than 0.01 were selected for further analysis. Three of them did not meet conditions of the Hardy-Weinberg equilibrium. Table 2 shows descriptive statistics of single nucleotide variants (SNV) used in the study.

Allele frequency analysis showed that rs686141T>C genome variant found in *NALCN* gene showed statistically significant difference

Table 1. Descriptive statistics of the study groups

Sex	Case group (n = 253), (%)	Control group (n = 41), (%)	Total (N = 294), (%)
Female	120 (47.43)	25 (60.98)	145 (49.32)
Male	133 (52.57)	16 (39.02)	149 (50.68)

Table 2. SNV statistics of 65 genome variants used in analysis

SNV	Counts (%)
Intronic variants:	33 (53.23)
UTR 3' variants	28 (84.84)
UTR 5' variants	5 (15.16)
Exonic variants:	29 (46.77)
Synonymous	11 (37.93)
Nonsynonymous	17 (58.62)
Stopgain	1 (3.48)

in relative alternative allele frequency between the study groups. Relative allele frequency of rs686141T>C variant was 0.27 in the alcohol drinker group and 0.38 in the non- drinker group (OR = 0.60 (CI 95% 0.37–0.98), $p = 0.0408$).

After the evaluation of genotype frequencies in the study groups, only one genome variant rs6354C>A in the *SLC6A4* gene showed statistically significant difference between the study groups. Variant genotype distribution (CC/CA/AA) was 9/72/172 in the case group and 5/7/29 ($p = 0.0264$) in the control group.

Table 3. Table of results of allele frequency evaluation in the study groups

Gene	SNV	Variant	Case group MAF*	Control group MAF*	*p* value†	OR‡
ADH5	rs7669660	NM_000671:c.*966T>C	0.08	0.10	0.5257	0.77
ADH5	rs11547772	NM_000671:c.*775T>G	0.05	0.05	0.8938	0.93
ADH5	rs6827292	NM_000671:c.*574T>C	0.03	0.05	0.4919	0.68
ADH5	rs1803037	NM_000671.4:c.*417G>A	0.05	0.05	0.8938	0.93
ADH4	rs1042364	NM_000670.4:c.*19A>G	0.34	0.24	0.0982	1.57
ADH4	rs1126673	NM_000670.4:c.1120G>A	0.38	0.33	0.3833	1.25
ADH4	rs1126672	NM_000670.4:c.1051C>T	0.34	0.24	0.0982	1.57
ADH4	rs1126671	NM_000670.4:c.925A>G	0.38	0.33	0.3653	1.26
ADH7	rs284787	NM_000673:c.*749C>T	0.24	0.28	0.3738	0.79
ADH7	rs3805331	NM_000673:c.*373T>C	0.05	0.06	0.6708	0.81
ADH7	rs971074	NM_000673.4:c.690G>A	0.13	0.15	0.7696	0.91
ADH7	rs17537595	NM_000673:c.-40T>C	0.16	0.15	0.7518	1.11
OPRM1	rs6912029	NM_000914:c.-172C>A	0.04	0.05	0.5588	0.72
OPRM1	rs1799971	NM_000914.4:c.118A>G	0.07	0.07	0.9988	1.00
OPRM1	rs563649	NM_001145287:c.-3004G>A	0.10	0.10	0.9836	0.99
OPRM1	rs650245	NM_001145286:c.*4A>G	0.08	0.10	0.6153	0.82
IPCEF1	rs9479767	NM_001130699:c.*4435T>C	0.46	0.54	0.1777	0.73
IPCEF1	rs17277929	NM_001130699:c.*3050T>C	0.09	0.07	0.5631	1.30
IPCEF1	rs2236256	NM_001130699:c.*2523G>T	0.46	0.54	0.1777	0.73
IPCEF1	rs2236259	NM_001130699:c.*2070T>C	0.46	0.54	0.1777	0.73
NPY	rs16139	NM_000905.3:c.20T>C	0.06	0.02	0.1963	2.52
DPYSL2	rs708621	NM_001197293.2:c.1821T>C	0.30	0.26	0.4142	1.25
DPYSL2	rs1058332	NM_001197293:c.*1071G>A	0.10	0.05	0.1586	2.09
DPYSL2	rs920633	NM_001197293:c.*1557A>G	0.14	0.09	0.1575	1.79
DPYSL2	rs17666	NM_001197293:c.*2236A>G	0.29	0.29	0.9738	1.01
ALDH1A1	rs8188000	NM_000689:c.*455T>C	0.07	0.11	0.2225	0.62
ALDH1A1	rs13959	NM_000689.4:c.225C>T	0.42	0.35	0.2507	1.33

Table 3. (continued)

Gene	SNV	Variant	Case group MAF*	Control group MAF*	*p* value†	OR‡
GAD2	rs2236418	NM_000818:c.-243A>G	0.21	0.21	0.9968	1.00
CYP2E1	rs6413419	NM_000773.3:c.535G>A	0.01	0.00	0.2840	NA
CYP2E1	rs2515641	NM_000773.3:c.1263C>G	0.13	0.09	0.2695	1.58
ANKK1	rs17115439	NM_178510.1:c.255T>C	0.32	0.25	0.2264	1.39
ANKK1	rs4938013	NM_178510.1:c.453A>C	0.33	0.26	0.2061	1.41
ANKK1	rs7118900	NM_178510.1:c.715G>A	0.16	0.12	0.3990	1.35
ANKK1	rs4938016	NM_178510.1:c.1324G>C	0.36	0.29	0.2126	1.38
ANKK1	rs2734849	NM_178510.1:c.1469A>G	0.48	0.59	0.0719	0.65
ANKK1	rs2734848	NM_178510.1:c.1683C>T	0.18	0.15	0.4843	1.26
HTR3B	rs1176744	NM_006028.4:c.386A>C	0.28	0.29	0.7644	0.92
HTR3B	rs17116138	NM_006028.4:c.547G>A	0.04	0.05	0.5588	0.72
HTR2A	rs9595552	NM_001165947:c.*1542T>C	0.07	0.06	0.8508	1.10
HTR2A	rs6314	NM_000621.4:c.1354C>T	0.07	0.06	0.7846	1.14
HTR2A	rs6313	NM_000621.4:c.102C>T	0.32	0.37	0.3725	0.80
NALCN	rs8922	NM_052867.2:c.*1454T>G	0.22	0.22	0.9656	0.99
NALCN	rs682767	NM_052867.2:c.*1018T>C	0.38	0.45	0.2296	0.75
NALCN	rs682666	NM_052867.2:c.*946C>T	0.39	0.45	0.2578	0.76
NALCN	rs9557581	NM_052867.2:c.*931A>G	0.38	0.45	0.2296	0.75
NALCN	rs1289556	NM_052867.2:c.4416A>C	0.38	0.33	0.4210	1.23
NALCN	rs17677552	NM_052867.2:c.3714C>T	0.37	0.30	0.2721	1.33
NALCN	**rs686141**	**NM_052867.2:c.3570T>C**	**0.27**	**0.38**	**0.0409**	**0.60**
CHRNA3	rs660652	NM_000743:c.*1114T>C	0.36	0.33	0.5473	1.16
CHRNA3	rs472054	NM_000743:c.*952T>C	0.36	0.33	0.5536	1.16
CHRNA3	rs578776	NM_000743:c.*546C>T	0.25	0.24	0.8608	1.05
CHRNA3	rs1051730	NM_000743.4:c.645C>T	0.38	0.43	0.3940	0.81
CHRNA3	rs8040868	NM_000743.4:c.159A>G	0.42	0.46	0.4470	0.83
CHRNA3	rs8192475	NM_000743.4:c.110G>A	0.03	0.04	0.8898	0.92
SLC6A4	rs3813034	NM_001045:c.*670T>G	0.43	0.48	0.4897	0.85
SLC6A4	rs1042173	NM_001045:c.*463T>G	0.43	0.48	0.4897	0.85
SLC6A4	**rs6354**	**NM_001045:c.-922G>T**	**0.18**	**0.21**	**0.5214**	**0.83**
COMT	rs4633	NM_000754.3:c.186C>T	0.46	0.51	0.3659	0.81
COMT	rs4680	NM_000754.3:c.472G>A	0.46	0.51	0.3659	0.81
COMT	rs769224	NM_000754.3:c.597G>A	0.04	0.07	0.1405	0.50
COMT	rs165599	NM_000754.3:c.*522G>A	0.35	0.44	0.1277	0.69
COMT	rs165728	NM_000754.3:c.*764C>T	0.11	0.17	0.1193	0.60

* MAF – Minor Allele Frequency

† *p* value – Pearson's Chi squared test *p* value

‡ OR – Odds Ratio

Statistically significant results are bolded

DISCUSSION

In this study, we examined the association of AUD with genome variants found in genes related to various enzymes, that are responsible for the metabolism of ethanol, neurotransmitters, or function of the receptors. Our data revealed novel genome variants in the association of *NALCN* and *SLC6A4* genes with AUD. rs686141T>C variant has never been studied before in similar AUD-related studies. Meanwhile, rs6354C>A variant was known but never studied more extensively. As a further matter, *NALCN* and *SLC6A4* genes were considered responsible for depressive phenotype and other psychiatric diseases.

In similar studies, it was hypothesized that the aetiology of both psychiatric diseases and AUD was related to a dysfunctional serotonergic system (7, 8). Serotonin is a monoamine known to affect anxiety, cognition, reward, emotion, drug responses, and stress (9, 10). The role of serotonin in alcohol consumption has been studied in animal models. The serotonergic system has been found to have only a minor role in mediating sensitivity to high doses of alcohol, but to be crucial for the development of alcohol reinforcement (8, 11). A study by Jiekun Yang and Ming D. Li showed that haplogroup formed by rs6354C>A, rs25528C>A, rs2066713C>T, rs8071667A>G, and rs16965623T>C showed a marginal association with AUD under the additive model ($P = 0.005$) (12).

Recent Genome Wide Association Study (GWAS) performed in a group of 2322 individuals demonstrated significant SNV (rs17484734G>A) located in the *NALCN* gene and a high risk of AUD (13). *NALCN* is a protein that contributes to the resting membrane potential in these neurons by eliciting a depolarizing current to counterbalance the hyperpolarizing current (14, 15). Changes in this system might be associated with substance addiction and AUD. A rodent study showed that mice that carry a hypomorphic mutation in the *Unc-79* gene (one of the *NALCN* subunits) voluntarily consume more ethanol than wild-type mice (16).

The identification of novel genome variants and AUD is important to improve our ability to predict the risks and treatment responses, to develop new treatments and screening techniques.

CONCLUSIONS

We analysed 23 genes associated with AUD and identified two novel genome variants (rs686141T>C and rs6354C>A). The study shows that genome analysis is an important tool in AUD research. The results supplement known information about genes associated with AUD.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

ETHICAL APPROVAL

All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the Vilnius Regional Research Ethics Committee (No. 158200-05-329-79. date: 2011-05-03) and with the 1964 Declaration of Helsinki and its later amendments.

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Karolis Baronas, Tautvydas Rančelis, Aidas Pranculis, Ingrida Domarkienė, Laima Ambrozaitytė, Vaidutis Kučinskas

NAUJI SU AVS SUSIJĘ GENOMO VARIANTAI LIETUVOS KOHORTOJE

Santrauka

Įžanga. Alkoholio vartojimo sutrikimas (AVS) – tai lėtinė atsinaujinanti nervų sistemos liga, kuri pasižymi alkoholio vartojimo priklausomybe, kontrolės praradimu jį vartojant ir neigiama emocine būsena, kai jis nevartojamas (1). Piktnaudžiavimas alkoholiu tiesiogiai veikia žmogaus fizinę, psichologinę sveikatą ir socialinį gyvenimą. Pasaulio sveikatos organizacijos duomenimis, Lietuva yra pirmaujanti šalis pasaulyje pagal grynojo alkoholio suvartojimą, tenkantį vienam žmogui (2). Dėl šių priežasčių pasirinktas tyrimo tikslas – identifikuoti naujus genomo variantus, susijusius su AVS Lietuvos kohortoje.

Tiriamieji ir metodai. Tyrimo metu analizuoti 294 lietuvių kilmės asmenų, kurie pagal alkoholio vartojimo įpročius buvo suskirstyti į vartojančių ir nevartojančių grupes, duomenys. Genotipavimas atliktas vieno nukleotido polimorfizmais paremto lyginamosios genomo hibridizacijos metodu, naudojant Illumina HiScanSQ™ genetinį analizatorių.

Rezultatai. Tyrimo rezultatai parodė, kad *NALCN* geno variantas rs686141T>C yra labiau paplitęs nevartojančių alkoholio grupėje, palyginti su alkoholį vartojančia grupe (santykinis alelio dažnis atitinkamai 0,38 ir 0,27, šansų santykis (ŠS) = 0,60 (pasikliautinas intervalas (PI) 95 % 0,37–0,98), *p* reikšmė = 0,0408). O rs6354C>A *SLC6A4* geno varianto genotipų pasiskirstymo grupėse analizė parodė statistiškai reikšmingą skirtumą tarp alkoholį vartojančių ir nevartojančių asmenų grupių (genotipų pasiskirstymas atvejo grupėje: 9/72/172 (CC/CA/AA) ir kontrolinėje grupėje: 5/7/29, *p* reikšmė = 0,0264).

Išvados. Atlikus 23 su AVS asocijuotų genų analizę nustatyti du genomo variantai (rs686141T>C ir rs6354C>A), kurie iki šiol nebuvo siejami su AVS. Tyrimas rodo, kad tolimesni genominiai tyrimai yra svarbi priemonė tiriant AVS. Gauti rezultatai papildo iki šiol gautą informaciją apie su AVS asocijuotus genus.

Raktažodžiai: alkoholio vartojimo sutrikimas, Illumina HiScanSQ, genotipavimas, *NALCN*, *SLC6A4*