DOI 10.18699/SBB-2022-223

Molecular mechanisms of cardio- and cerebrovascular comorbidity: from experimental analysis of structural and epigenetic variations in the human genome to post-GWAS analysis of genetic correlations between diseases

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Key words: comorbidity, hypertension, coronary heart disease, stroke, genetic variants, DNA methylation, genetic correlations, LCV analysis

Motivation and Aim: Common and often combined cardio- and cerebrovascular diseases (CCVDs), including arterial hypertension (AH), coronary heart disease (CHD), and cerebral stroke (CS), are the main cause of mortality and disability worldwide [1]. Despite the obvious progress in the genetics of human CCVDs, investigation aimed at studying the molecular genetic mechanisms of the formation of cardio- and cerebrovascular comorbidity are rare. The aim of our study was the molecular genetic, epigenetic and bioinformatic analysis of the formation of comorbid cardio- and cerebrovascular diseases.

Methods and Algorithms: A total of 2685 unrelated Russians (890 healthy individuals and 1795 patients with various CCVDs and their combinations) from Kursk region was examined; written informed consent was obtained from all participants prior to entering the study [2, 3]. The study was approved by the Ethics Committee of Kursk State Medical University. When selecting genes and polymorphisms for genotyping, the main emphasis was placed on the genes of the antioxidant and detoxification systems, as well as known CCVDs candidate genes that showed an associations with the risk of hypertension, coronary heart diseases and stroke in previous studies; the amount of 34 genetic markers were genotyped. A log-additive regression model was used to interpret the results of the analysis of associations. The correction for multiple comparisons was carried out using an adaptive permutation test in the PLINK program. The Bonferroni correction was introduced for the number of comparison groups. DNA methylation analysis was performed in 84 healthy individuals and 128 patients with various CCVDs and their combinations. To assess the status of DNA methylation in peripheral blood cells, the promoter regions of the gene *TXNRD1* (3 CpG-site), *GSTP1* (2 CpG-site) and *GCLM* (4 CpG-site) and exons 4–6 *MPO* (3 CpG-site) were selected [4]. The Mann-Whitney test with the Bonferroni correction for multiple comparisons was used for comparative analysis of the methylation levels. The Complex-Traits Genetics Virtual Lab (https://vl.genoma.io/) was engaged for bioinformatic evaluation of genetic correlations between various cardio- and cerebrovascular phenotypes (by LD score method), and also to analyze the mechanisms of formation of genetic correlations (LCV, or analysis of models of the latent causal variable) [5].

Results: The arterial hypertension was associated with genetic variants *FGB* rs1800790 (OR = 0,81; P_{perm} = 0,007) and *CYP1A2* rs762551 (OR = 0,81; P_{perm} = 0,005). *PON2*

rs7493 showed an association exclusively with the comorbid phenotypes: AH+CHD (OR = 1,32; P_{perm} = 0,002), AH+CS, AH+CHD+CS (OR = 1,76; $P_{perm} = 1{,}00\times10^{-6}$). At the same time, *TXNRD1* rs1128446 was associated solely with cerebrovascular phenotype (OR = 1,83; $P_{perm} = 1{,}00\times10^{-6}$). We observed an increase in the level of *MPO* gene methylation in healthy individuals (35,76 %) compared with patients suffering from AH (28,74 %), AH+CHD (26,29 %), AH+CS (24,27 %), AH+CHD+CS (22,1 %); P-levels were $P_{bonf} = 8{,}56\times10^{-4}$, $P_{bonf} = 1{,}39\times10^{-7}$, $P_{bonf} = 4{,}32\times10^{-10}$ and $P_{bonf} = 2{,}70\times10^{-10}$ consequently. To analyze the genetic correlations between different diseases as "input data", there were used the results of GWASs for three diseases (arterial hypertension, ischemic heart disease, cerebral stroke), presented in UKBB. Based on bioinformatic post-GWAS analysis of UKBB data in the Complex-Traits Genetics Virtual Lab statistically significant genetic correlations were established for the following phenotype pairs: AH vs CHD (rG = 0,3701; P = 0,0012); CS vs CHD (rG = 0,785; P = 0,0005). At the same time, the phenotypes of AH vs CS did not correlate with each other. Statistically significant latent causal variable model was discovered exclusively for the phenotypes CHD vs CS, supposing that the bigger part of genetic component of CHD causes CS (GCP = 0,62; P_{LCV} = 0,03), while genetic correlations between AH and CHD are mainly caused by horizontal pleiotropy.

Conclusion: Thus, the structural and epigenetic variability of the human genome is one of the most important factors in the formation of complex, comorbid cardio- and cerebrovascular phenotypes.

Acknowledgements: The study is supported by Kursk State Medical University.

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