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Reply

Reply to Comment on Josef H. Finsterer, *et al.* "Polymorphism in Genes Encoding HSP40 Family Proteins is Associated With Ischemic Stroke Risk and Brain Infarct Size: A Pilot Study. Journal of Integrative Neuroscience. 2024;23(12):211"

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Thank you very much for your interest in our research and for the comments.

Indeed, our study established an association between polymorphisms of individual genes encoding members of the heat shock protein (HSP) family 40 family and the risk of developing, and the clinical manifestations of, ischemic stroke (IS) [1]. Our study was a pilot project in which a genetic epidemiological study in a case-control design was used to analyze associations of polymorphic loci. That was followed by a functional annotation of genetic variants using a range of bioinformatics tools that are widely used to interpret the functional role of single nucleotide polymorphisms (SNPs) and study their potential involvement in the molecular mechanisms of the disease [2–7].

We would like to point out that our work was an association study and did not conclude causal effects of *HSP40* SNPs in IS. The nature of the discovered associations has yet to be elucidated, and further experimental studies will certainly be able to shed light on the causal relationship between HSP40 family proteins and IS. As is standard in genetic epidemiology, association studies represent the first step in hypothesis generation that can guide functional follow-up *in vitro* or *in vivo* studies. This was clearly acknowledged in our discussion section.

In our work, we found associations of SNPs in *HSP40* genes in a large sample of patients and controls. That sample was described in detail in our previous articles [8–13]; it is one of the largest research samples in Central Russia and included more than 2500 people, of whom 1306 are patients with IS. That sample size ensured the high power for genetic calculations necessary to obtain unbiased representative results in the field of association studies of complex human diseases [14]. In this regard, the associations we found do not raise any doubts. Furthermore, to account for biological heterogeneity in the cohort (such as age, sex, smoking), we included those variables as covariates in the logistic regression model, which complied with the current methodological standards for genetic-epidemiological research and ensured that our results were not confounded by known non-genetic risk factors.

Moreover, the associations we obtained were replicated (confirmed) using the Cerebrovascular Disease Knowledge Portal (CDKP [15]), which combines data (summary statistics) from association studies conducted around the world. Specifically, two independent genome-wide association studies (GWAS) reported associations between rs7189628 DnaJ heat shock protein family (Hsp40) member A2 (*DNAJA2*) and stroke [16,17]; as for rs2034598 *DNAJA2*, it was found to be associated with the white matter hyperintensities in cerebral small vessel disease by Persyn *et al.* [18]. In addition, rs6500605 *DNAJA3* was found to be associated with post-stroke functional outcomes (Modified Rankin Scale 0–1 *vs* 2–6) in a separate study [19].

Currently, the GWAS catalog contains information on more than 600 IS risk loci identified by genome-wide association studies [20]. Hundreds of IS risk loci have also been identified by the candidate approach [21–30]. However, the use of a candidate approach in the association analysis does not require the inclusion of SNPs identified in previous association studies as potential risk factors. The candidate approach remains a widely accepted strategy for investigating biologically plausible genes (such as those involved in proteostasis and stress response) that may not reach genome-wide significance thresholds due to limited sample sizes or effect sizes below detection power in GWAS meta-analyses.

The functional effects of SNPs are not limited to the direct influence of genotypes on the expression level of the corresponding genes. (The expression level, by the way, is highly tissue-specific and cannot always be measured in patients in tissues of high pathogenetic significance for the disease under study.) However, SNPs can actualize their diverse functional role, for example, by binding to transcription factors [4,31–33], influencing histone modifications [7,34], and influencing the expression level of other genes through binding to quantitative trait loci [3,6,35].

Finally, as indicated in **Supplementary Table 1** [1], there were no individuals with diabetes in the patient or control groups. Hyperlipidemia and atrial fibrillation are phenotypes that may also be determined by the genes we studied. Since only independent risk factors can be included in the regression model for association analysis (stroke and hyperlipidemia, and stroke and atrial fibrillation are phenotypes that may be codetermined by the genes we studied), we included sex, age, and smoking as covariates—independent risk factors for IS.

We hope this detailed response clarifies our study design, findings, and rationale. Ultimately, the aim of our work was to open new directions in understanding IS susceptibility.

Author Contributions

OYB designed the research study. KAK, DEG, VMP and EAP performed the research. AVP analyzed the data. KAK and OYB wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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