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EVALUATION OF FUNCTIONAL EFFECT OF GENES AND THEIR VARIANTS ASSOCIATED WITH SCHIZOPHRENIA

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Prediction of the functional role of the genetic variants in Schizophrenia (SCZ) using the bioinformatics toolkit showed that decreased levels of *CPLX2* gene are associated with the rs1366116*T allele and rs3892909*C alleles of the same gene. On the contrary, rs4940456 is not associated with changes in the *CPLX4* expression level, but the other SNPs linked to it correlate with the increased expression of the *LMAN1*. Minor allele of rs1049171-linked SNP rs17106725*T is associated with the increased *CRMP4* expression. The expression of the *STK32A* gene is also increased in patients with SCZ, though LD analysis did not reveal genetic variants linked to rs1049171. Thus, the carriage of rs1049171*G does not have any impact on the *STK32A* expression in SCZ. Finally, rs1366116 and rs3892909 of the *CPLX2*, rs4940456 of the *CPLX4* and rs1049171 of the *CRMP4* genes might be indirect markers for the gene expression changes in patients with SCZ.

Keywords: synaptic plasticity, polymorphism, gene expression.

Introduction. Schizophrenia (SCZ) is a complex mental disorder characterized by gene–environment interplay [1]. The "synaptic theory" of SCZ says that the alterations in synaptic plasticity can underlie the changes in the brain functioning and neural network and cause cognitive symptoms in SCZ [2]. The cognitive system alterations are observed during both positive and negative periods of SCZ and lead to the social exclusion of the individual suffering from SCZ [2].

Thus, we focused our research on studying the genes, encoding the direct and indirect regulators of synaptic plasticity in order to understand if the cognitive alterations in SCZ are genetically determined. Our previous genetic studies indicated three genes (complexin 2 – *CPLX2,* complexin 4 – *CPLX4,* collapsin response mediator protein 4 – *CRMP4*) with their variants associated with SCZ: *CPLX2* (rs1366116, rs3892909), *CPLX4* (rs4940456) and *CRMP4* (rs1049171) [3, 4]. Interestingly, the analysis of available databases showed that the rs1049171 besides *CRMP4* also spans *STK32A* gene, encoding serine/threonine kinase 32A. Thus, the results obtained for the *CRMP4* were also interpreted for *STK32A*.

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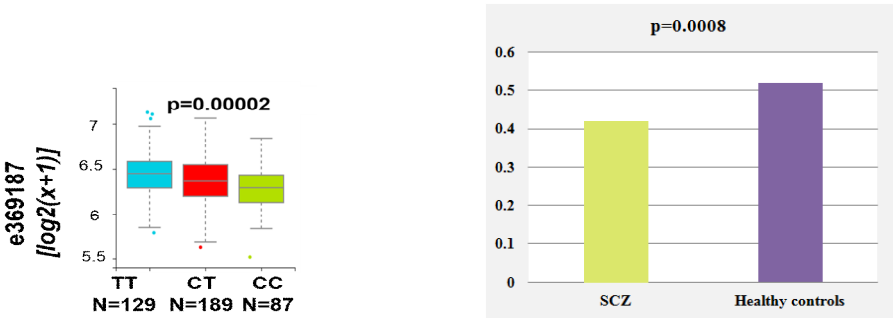
In this study we sought to assess the potential functional effect of the mentioned polymorphisms using bioinformatics tools and publicly available data resources.

Materials and Methods. For the assessment of the potential functional effect of the polymorphisms mentioned above, we have analyzed the association of their minor alleles with the expression levels of the *CPLX2, CPLX4, CRMP4* and *STK32A* genes using bioinformatics tools. The analysis of the BrainSeq database was performed to compare genotype-dependent expression levels of these genes in the dorsolateral-prefrontal areas of the brain [5]. Further, the Linkage Disequilibrium (LD) and Quantitative Trait Loci (QTL) analyses, as well as Variant Effect Prediction tools, were used to identify the SNPs linked to the genetic variants associated with SCZ.

Results and Discussion. According to the data obtained, only rs3892909 of *CPLX2* among the SCZ-related SNPs has been associated with the alterations of *CPLX2* expression level in patients with SCZ (p=0.00002) (Fig. 1, a). Moreover, the rs3892909*T minor allele carriage is associated with the decreased expression levels of *CPLX2,* which coincides with our own results of the genetic analysis showing the protective role of rs3892909*T in the pathogenesis of SCZ [3] (Fig. 1, b).



a b

Fig. 1. a) Correlation between the carriage of the rs3892909*T minor allele and *CPLX2* expression in patients with SCZ; b) rs3892909*T minor allele frequency in patients with SCZ and healthy controls.

The BrainSeq analysis of the rest of the genes did not show significant differences in the expression associated with the studied SNPs. However, the expression of *CRMP4* was significantly increased in patients with SCZ compared to healthy controls.

LD data analysis of the SNPs has detected 15 SNPs linked to both rs1366116 and rs3892909 of *CPLX2* gene. According to the Variant Effect Prediction [6] database, three of them, including rs3892909, are regulatory SNPs and are located in the flanking regions of the *CPLX2*. LD analysis shows that the higher frequency of rs1366116*T and the lower frequency of rs3892909*T are associated with the decreased expression levels of *CPLX2*. Interestingly, the *CPLX2* expression level changes with the age and reaches its maximum in late childhood (Fig. 2), when the early symptoms of the SCZ are first observed. It can be suggested, that the SNPs decreasing the *CPLX2* expression levels can induce alterations in synaptic plasticity of the brain, especially during childhood, which can be a risk factor for the SCZ development.

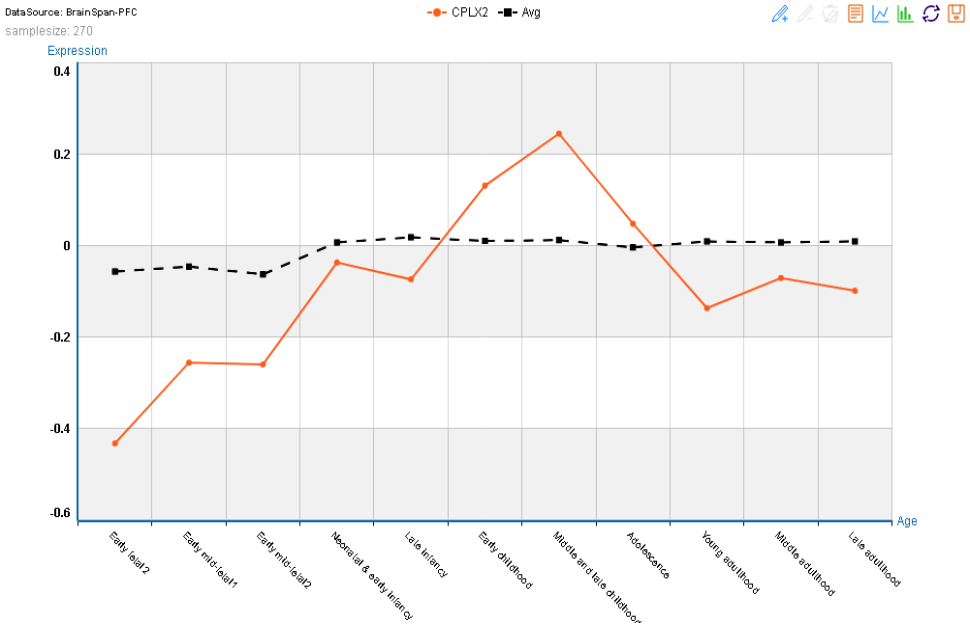


Fig. 2. *CPLX2* expression dynamics depending on the individual's age.

QTL data analysis has shown that rs4940456 is not associated with the *CPLX4* expression level changes. On the other side, the LD analysis showed that the mentioned SNP is linked to the other SNPs (see Table) associated with increased expression of *LMAN1* (Fig. 3), encoding mannose-binding lectin (MBL), a component of the lectin pathway of the complement system. This finding is in line with previously obtained results implicating the lectin complement pathway in the pathogenesis of SCZ [7].

LD analysis of the CPLX4 rs4940456 SNP

SNP	Measures of linkage disequilibrium with rs4940456	
	D′	R2
rs11275112	0.9959	0.6696
rs9973070	0.9957	0.6175
rs7243416	0.9949	0.6567

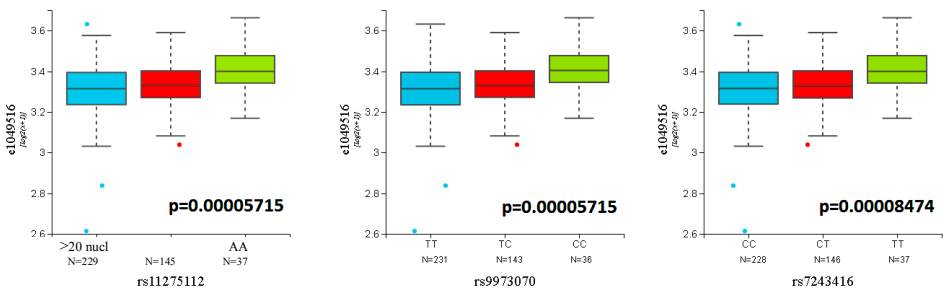


Fig. 3. Correlation between the carriage of SNPs: rs11275112, rs9973070, rs7243416, and *LMAN1* expression.

QTL analysis of the rs1049171 of *CRMP4* also did not show an association between the genotypes of the SNP and expression level of *CRMP4,* but the LD analysis has resulted in the rs17106725 polymorphism linked to the studied SNP. The linkage was observed between rs1049171*G and rs17106725*T alleles (G-T haplotype frequency in CEU is 0.8). In turn, the rs17106725*T allele is associated with the increased expression of *CRMP4* (Fig. 4).

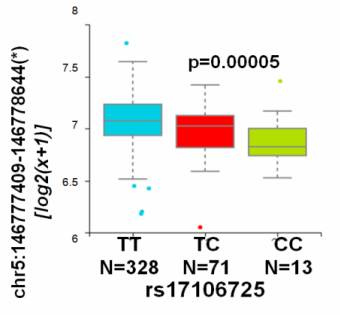


Fig. 4. Correlation between the carriage of the rs17106725*T minor allele and *CRMP4* expression in patients with SCZ.

The results of this analysis showed that the carriage of the rs17106725*T and rs1049171*G alleles is associated with the increased expression of *CRMP4* in SCZ. The expression of the *CRMP4* gene reaches its maximum in the early embryonic period and decreases sharply in the postembryonic period (Fig. 5).

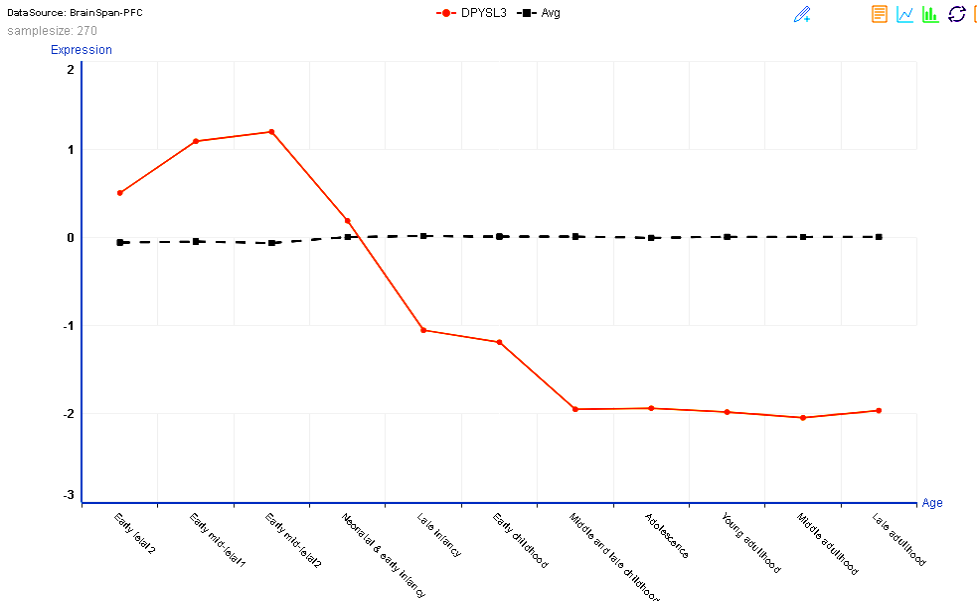


Fig. 5. *CRMP4* expression dynamics depending on the individual's age.

The expression of *STK32A* gene was also increased in patients with SCZ (Fig. 6). However, unlike *CRMP4* expression, LD analysis did not reveal SNPs in

STK32A associated with the rs1049171. This allows us to conclude that the carriage of the rs1049171*G does not have any impact on the *STK32A* expression in SCZ.

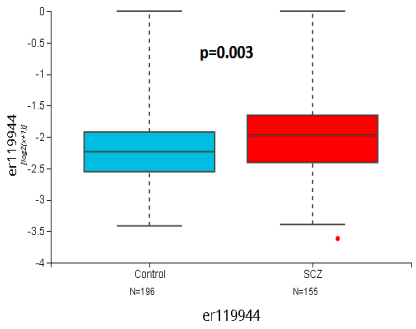


Fig. 6. *STK32A* gene expression.

Conclusion. Summarizing the results of the bioinformatics analysis of the functional role of the studied SNPs, we can conclude that the rs1366116 and rs3892909 of the *CPLX2*, rs4940456 of the *CPLX4* and rs1049171 of the *CRMP4* genes might be indirect markers of gene expression alterations in patients with SCZ.

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