POSSIBLE EFFECT OF SNP TATA-BOXES OF HUMAN ERYTHROPOIESIS GENE PROMOTERS ON COGNITIVE DISORDERS

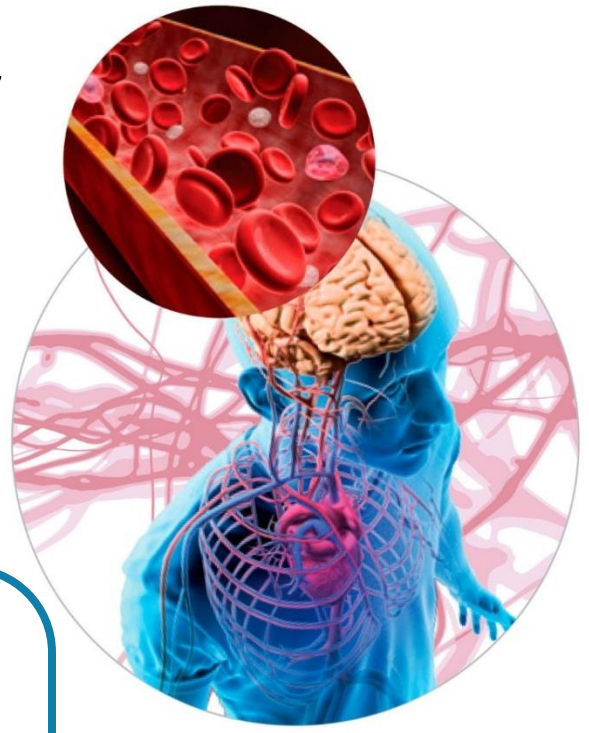
Keywords: *TATA-binding protein, TATA-box, TBP/TATA-affinnity, erythropoiesis, single nucleotide polymorphism, cognitive disorders*

Introduction

In recent years, there is increasing evidences that various forms of anemia, changes in the quantity and quality of blood cells may be involved in the pathogenesis of various cognitive disorders accompanying Alzheimer's and Parkinson's diseases, depression of various degrees, etc [1].

~7% of the world population have hereditary aberrations in the synthesis of hemoglobin, that mean these are the most prevalent monogenic disorders (WHO) [2].

In addition to erythroid cells, hemoglobin has been shown to be widely expressed in non-erythroid cells, including neurons of different parts of the brain [3]. *The morbidity and also prevalence of anemia and elevated hemoglobin levels increase with age, which is a heavy burden for society.*



We analyzed in silico and in vitro unannotated SNPs in TATA boxes of human genes involved in erythropoiesis. Experimental verification in vitro using the method of electrophoretic mobility shift assay (EMSA) showed the correspondence of prognosis and experimental data. The estimates obtained of the effect of TATA box SNP markers on the formation of TBP/TATA complexes make it possible to consider some SNP markers of erythroid genes as markers of cognitive disorders.

All this determines the relevance of the problem and the goal of the work, which is to study the effect of SNP TATA boxes of human gene promoters on erythropoiesis and cognitive disorders

MATERIALS:

- *ODNs identical to ancestral and minor alleles of the selected SNPs*
- *Recombinant full-length human TBP*

METHODS:

- *SNP-TATA_Z-TESTER (WEB-SERVICE)*
- *EMSA (Savinkova et al., 2013; doi: 10.1371/journal.pone.0054626)*

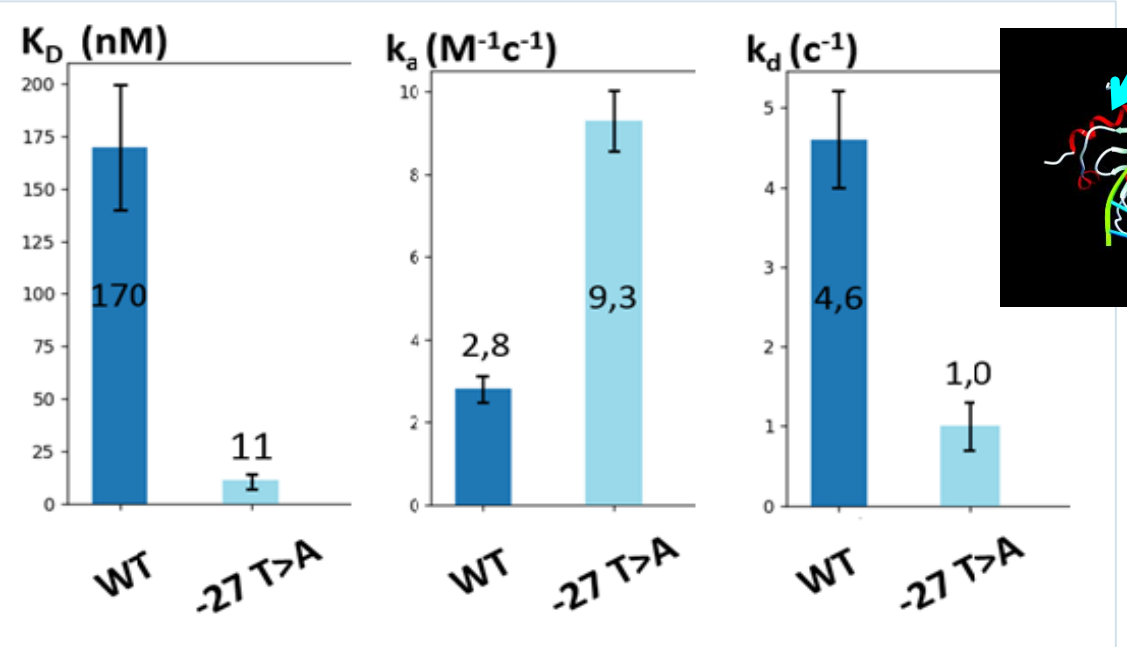
Results and Discussions

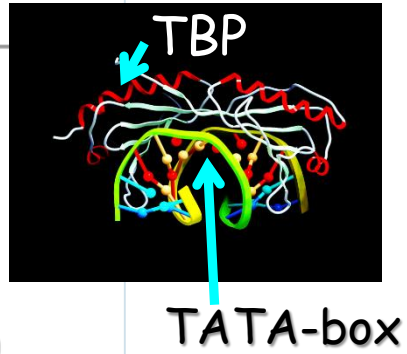
We analyzed unannotated SNPs in TATA boxes of human globin genes - HBZ, HBB, HBD, HBG1, as well as genes encoding enzymes involved in heme biosynthesis - ALAS1, CA1, EPOR, GYPC and others involved in erythropoiesis, a total of 161 SNPs 25 human genes. As a result of the analysis using the SNP-TATA_Z-tester Web service, 45 candidate SNP markers were predicted for 15 of 25 genes that can reliably change the affinity of TBP to the TATA boxes of gene promoters participating in erythropoiesis, and an in silico prognosis of the effect of SNPs on affinity TBP interactions with TATA / TATA-like sequences.

As an example, let to consider the EPOR gene. It is known, the glycoprotein hormone EPO is the main regulator of red blood cell production and exhibits protective properties in cerebral ischemia [4]. To perform the function it binds to its receptor, EpoR, which dimerizes and activates, which leads to the launch of a cascade of genes responsibled for the proliferation, survival and differentiation of erythroid progenitor cells. While EpoR in the blood regulates the differentiation of red blood cells, in the brain it protects several types of neuronal cells from death, including A9 dopaminergic neurons (DA) of the Substantia Nigra (SN) and stores oxygen during hypoxia. It is especially important for neurons with an increased energy requirement. In addition to the regulation of erythropoiesis in hematopoietic tissues, where EPOR mutations are the cause of primary hereditary erythrocytosis and are found in 15% of all cases, erythropoietin is expressed in other tissues including the nervous system. For functioning, erythropoietin uses a homodimeric receptor (EpoR), which is also widely expressed in the nervous system. The main role of EpoR is to stimulate the proliferation of red blood cell precursor cells for their survival.

The interaction of TBP with minor and ancestral TATA-box alleles of the EPOR gene

Gene	Alleles: WT or mut	(5–3 (strands), 26 bp	Prediction		Experiments					
			$-\ln K_D$	δ	$-\ln K_D$	δ	K_D, nM	ka, $M^{-1}s^{-1}$ *10^3	k_d, s-1 * 10^{-4}	$t_{1/2}$, min
EPOR	WT	ctatttttgt	18.24		15.59		170±30	(2.8±0,3)	(4.6±0.6)	25±3
	-27t>A	ctatAttt	19.39	+1.15	18.33	+2.74	11±3	(9.3±0,7)	(1.0±0.3)	115±15





A 15-fold increase in affinity is caused by the SNP of -27T> A (rs1006576690) in the TATA box of the *EPOR* gene (K_D = 170 ± 30 nM and 11 ± 3 nM. K_D is presented as mean ± standard deviation; δ: the difference between the affinity of hTBP for an ODN with and without the SNP in its TATA box expressed as natural logarithms, δ = -ln (K_D,TATA,Mut) – [-ln (K_D, TATA)]; $t_{1/2} = \ln 2/k_d$.

This is accompanied by a 3.3-fold increase in the rate of formation of complexes (k_a) and a 4.6-fold increase in their lifetime (or half-life, $t_{1/2}$). Several studies have shown that cerebral ischemia leads to increased expression of Epo and EpoR to repair damage. Based on this, it can be assumed that substitutions in the TATA-like sequence of the *EPOR* gene promoter are -27T>A, -31C>A and -31C>G, leading to an increase in the affinity of the TBP/TATA interaction by 15.5, 8.1 and 2.1 times, which correlates the level of gene expression may indicate a degree of hereditary ischemic brain damage in carriers of minor alleles and may be candidate markers of this disease.

CONCLUSIONS

So, it is known that hemoglobin genes are expressed in astrocytes of the cortex and hippocampus, in oligodendroglia located in almost all areas of the brain, including the striatum, corpus callosum and medulla oblongata.

There are many reports of the association of hemoglobin metabolism disorders with symptoms of mental illness. It is shown that among children with anemia there are significantly more problems with behavior and low intelligence.

Therefore, based on the literature data and the computer-experimental results obtained it can be concluded that the identified candidate SNP markers of erythropoiesis disorders can be candidate SNP markers of cognitive and mental disorders also.

The information received is an additional resource in biomedical research, personalized medicine (P4 medicine), diagnostics and drug development

ACKNOWLEDGMENTS

The work was supported by the budget project No. 0324-2019-0042-c-01

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