Differential gene expression by RNA-seq data in brain structures of laboratory animals with aggressive and tolerant behavior

Anatoly O. Bragin, Nataly N. Kudryavtseva, Arcady L. Markel, Yuriy L. Orlov,

Institute of Cytology and Genetics, Lavrentyeva 10, 630090, Novosibirsk, Russia,
{ibragim,natnik,markel,orlov}@bionet.nsc.ru

Ekaterina V. Kulakova, Anastasia M. Spitsina

Novosibirsk State University, Novosibirsk, Russia,
kylakovaekaterina@gmail.com, anastasia.spitsina@gmail.com

Aggressive behavior is a complex behavior phenomenon having genetics and physiological roots. Basic studies have shown that the frequency and severity of aggression depends on the hereditary predispositions, previous experience of aggressive behavior and social context, provoking the demonstration of aggression. To study genetic component of aggressive behavior we used published data on genes expression (RNA-seq and microarrays) related to aggressive behavior in mouse and rat as well as in-house experimental data [1].

The research was intended to study the molecular genetics mechanisms of enhanced aggressiveness in comparison with tolerant behavior using two unique experimental models which were developed at the Institute of Cytology and Genetics SB RAS. One of them - grey rats (*Rattus norvegicus*), which have been subjected to selection during several generations in two directions - friendly, tolerant behavior towards man (tame gray rats) and increased aggressive behavior ("aggressors") The last rats demonstrated reinforced aggression not only towards man (in the glove test) but also towards conspecifics in intermale agonistic interactions. In other model in mice, increased aggressiveness is the result of repeated fighting experience in daily encounters that means the social “learning”. As a consequence of repeated aggression, the social and individual behaviors become pathological: such males attacked females or other mice demonstrating submissive behavior. The research aimed to elucidate the genetic and molecular mechanisms of hereditary defined (first model) and acquired (second model) increased aggressiveness using gene expression profiling by RNA-seq in different brain regions from aggressive and tame animals.

Studies of gene expression in brain on animal models have long traditions [2].

Affymetrix microarrays were used to detect differences in brain gene expression between two inbred mouse strains (C57BL/6J and 129SvEv) [3]. Some differentially expressed genes were found in chromosomal regions with known behavioral quantitative trait loci (QTLs). Thus, Kcnj9 which encodes for GIRK3, an inwardly rectifying potassium channel, was differentially expressed is located in a region where QTLs had been identified for locomotor activity, alcohol and pentobarbital withdrawal, open-field emotionality, and certain aspects of fear-conditioned behavior. The alignment of differentially expressed genes with a behavioral phenotype can be further examined using a variety of secondary analyses, e.g., examining if the genes cluster within known gene ontology categories or are part of a known protein-protein interaction network [4]. Selected genes can be grouped on the basis of common transcription factors and other regulatory elements.

The following brain areas involved in regulation of aggressive and tolerant behavior were examined: prefrontal cortex, ventral tegmental area containing the dopaminergic and opioidergic neurons and responsible for the positive reinforcement, midbrain raphe nuclei containing serotonergic neurons responsible for inhibitory control of aggression, and the amygdala regulating emotions. Proper selection of brain area is a key for cell sample preparation. Control of gene expression at the level of mRNA translation is significant for both neuron development and morphogenesis, as well as for the functioning of distinct gene networks in the mature cells of various brain regions.

After constructing differentially expressed gene lists we used set of tools for coexpression analysis to found characteristic features of gene network related to aggressive behavior. The use of computer technologies such as GeneNet and AndVisio [5] gives the possibility of reconstructing genetic networks - assemblies of coordinately functioning genes controlling biochemical, molecular-genetic, and physiological processes - based on the published data.

At the first step study included obtaining features of genes actively expressed in the brain using complex computer analysis, including methods and programs previously developed by the authors, such as the Affymetrix U133 database of the quantity of microarray probes [6] and ICGenomics for the functional annotation of genes [7]. We

reconstructed some gene networks and metabolic pathways determining role of molecular-genetic systems in realization of aggressive and tolerant behavior in varying conditions. Note that in scientific literature on genetics of aggressive behavior main conclusion underline multi-loci determination of aggressive behavior. There are no major genes reported.

Gene expression, i.e., the manifestation of its functions, in brain cells represents the basis of neuron functioning. Usually, the cell is characterized by the simultaneous expression of a small number of genes (about 5%) rather than all genes, which makes it possible to extract organ-specific groups of genes. To search genes with specific expression in the brain regions, we used Allen Brain Atlas and BioGPS databases containing the data of gene expression in a wide variety of tissues and organs. Gene expression was determined using Affymetrix U133 microarrays with probe quality filtration [6]. The probes with high expression (according to the ranking of the probes of all genes, 1%) and genes expressed in the brain regions (hypothalamus, prefrontal cortex, etc.) and not expressed in other organs (kidney, liver, smooth muscles, etc.) have been selected from the Affymetrix U133 microarray probes represented in the BioGPS database (http://biogps.org). After the deletion of duplicated microarray probes, 11830 genes (unique identifiers) have been selected [7]. Among these genes, 1801 genes (15%) were overexpressed in at least one region of the brain. The majority of the remaining genes (9253) were characterized by insignificant expression in any brain region. It should be noted that only protein-encoding genes in microarray were analyzed. At the next step 1% genes over-expressed in average in the studied brain regions relative to other organs were selected For the list of genes obtained, gene genomic surrounding, context structure of regulatory regions, overlapping with microRNAs, short non-coding transcripts in the opposite orientation, the number of exons, and evolutionary conservatism were analyzed. The functional annotation of 1382 genes with a high expression only in the brain was conducted by the DAVID software for the gene ontology analysis (http://david.abcc.ncifcrf.gov).

Interestingly, the presence of categories of protein transport, phosphoproteins, and nucleotide binding, rather than transcription should be mentioned. The categories of transmission of nerve impulses and neuron development were demonstrated, which is

expected for the brain regions. Almost half of the genes (45.7%) from the list are related to alternative splicing. RNA-seq analysis of differentially expressed genes in rat and mice confirmed presence of genes known as related to aggressive behavior, such as MaoA. We continue work on gene network reconstruction using RNA-seq experiments on additional mouse brain structures in contrast groups of laboratory animals using digital atlas of such structures [8].

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