Research Article

Characteristic Features of the Transcriptome in a Rat Strain with Audiogenic Epilepsy

Lyubov N. Chuvakova[1], Sergey Yu. Funikov[1], Artem I. Davletshin[1], Irina B. Fedotova[2], Mikhail B. Evgen'ev[1], Inga I. Poletaeva[2], and David G. Garbuz[1]*

[1]Engelhardt Institute of Molecular Biology RAS, Laboratory of the Molecular Mechanisms of Biological Adaptation, Moscow, Russian Federation
[2]Moscow State University, Biology Department, Chair of Higher Nervous Activity, Laboratory of Physiology and Genetics of Behavior, Moscow, Russian Federation
ORCID
David G. Garbuz; 0000-0002-5813-2220

Abstract. Audiogenic epilepsy (AE), developing in rodent strains in response to sound, is widely used as the model of generalized convulsive epilepsy, while the molecular mechanisms determining AE are currently poorly understood. The brain region that is crucial for AE development isthe inferior and superior colliculi (IC, SC). We compared IC-SC gene expression profiles in rats with different AE susceptibility using transcriptome analysis.The transcriptomes were obtained from the IC-SC of Wistar rats (with no AE), Krushinsky-Molodkina (KM) strain rats (100% AE susceptible), and "0" strain rats (with no AE) selected from F2 KM x Wistar hybrids for AE absence. KM gene expression displayed characteristic differences inboth of the strains that were not susceptible to AE. There was increased expression of a number of genes responsible for positive regulation of the MAPK signaling cascade, as well as of genes responsible for the production of interferon and several other cytokines. An increase in the expression levels of the*TTR* gene was found in KM rats, as well as significantly lower expression of the *Msh3* gene (involved in post-replicative DNA repair systems). AE was also describedin the 101/HY mouse strain with a mutation in the locus controlling DNA repair. The DNA repair system defects could be the primary factor leading to the accumulation of mutations, which, in turn, promote AE.

Keywords: udiogenic seizure, KM strain, transcriptome, *TTR* gene, *Msh3* gene, DNA repair

Corresponding Author: David G. Garbuz; email: dgarbuz@yandex.ru

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1. Introduction

Audiogenic epilepsy (AE, seizures, induced by loud sound) were first noted in the first laboratory rat colony (Wistar Institute), later the same trait was found in mice in the Leningrad laboratory of I. Pavlov. The audiogenic seizure fit has the specific pattern of expression – after the short latency (depending on strain used) after the switching on the loud (100-120 dB) sound seizure fit starts. The sound used could be either the tone of high frequency or the sound of the auditory bell. The fit starts by the "wild run"-the intense running and jumps of an animal. Later this stage was named "clonic run", as this reaction is not pure "running behavior", the involuntary sound induced seizure

component could be discovered during this stage (confirmed in two independent studies (see(1)). This “wild run” is the characteristic feature of AE – be it rat, mouse or hamster. In animals with high predisposition to AE the wild run stage is followed by clonic and later- tonic seizures involving all muscles of trunk and legs.

The AE as a pathology trait (and as the productive model of seizure state for antiepileptic drugs study) is now analyzed in several rodent strains. The Krushinsky-Molodkina (KM) strain selection (on the basis ofWistar population) started in 1940s and it had been the first rat strain selected for AE. Later GEPR and WAR strains were selected as well (2) and the audiogenic seizures being detected in other rat strains as well (e.g. - WAG/Rij). In 1990s the 12 generations of inbreeding were performed and KM strain is now the inbred the inbred status pf this strain being confirmed (3).

The genetics of AE is not clearly established – the diallel cross, performed in 1970s, using KM strain and several other rat strains (with different AE proneness) proved the inheritance of AE to be the polygenic recessive trait (1). Later, when KM strain was already inbred the digenic model with incomplete penetrance has been put forward to explain the control of audiogenic seizure fits(4). This model fits the data obtained: the theoretically expected distributions of the character in offspring of different crosses do not differ significantly from those observed in experiments.

As several dozen years elapsed from the start of KM selection on the basis ofWistar outbred strain, it became obvious that Wistar rats are not the ideal control for AE studies in Km rat. The where two main reasons for this conclusion - , first, because mutations in both strains, not connected directly with AE, could nevertheless influence the trait, and second – about 20% of Wistar rats demonstrated AE of low level. The breeding of the new strain – named ‘0” was created with main features of it to be mentioned here (5). The definite proportion (20-30%) of “0” rats demonstrates rather “robust” lack of AE in response to loud sound during their life time, while the rest show low degree of fit severity and are regularly discarded from further breeding. In parallel to this activity some changes occurred in the status of Wistar rats. Wistar line after embryo transfer (performed in PoushchinoDepartment of Experimental Biology with Vivarium) now are successfully breeding. But something change happened in the Wistar rats genome as the result of this procedure - now no Wistar rat demonstrate AE–type reaction to sound. Thus, the study of gene expression in KM rats could be compared with rats of two genotypes (‘0” and “Wistar”), which makes the results more conclusive.

Fig. 1 demonstrates the list of brain structures, involved in AE seizure development. The intense excitation, aroused in response to sound spreads along the brain stem up to corpora quadrigemina, colliculi inferior being the first and colliculi superior –

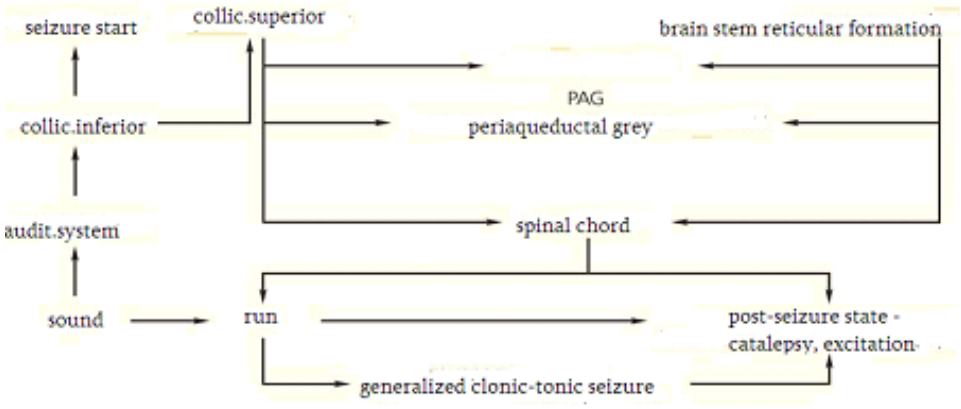

Figure 1: The general pattern of brain structures involved in AE stages expression (see (10)).

the second structure, crucial for the AE seizure development(6, 7). The neocortex and hippocampus don't participate this process, while changes in basal ganglia are now under intense investigation (8). The EEG studies which started using KM rats in 1950s(9)and afterwards proceeded by C.L. Faingold in GEPR strains(10) stated that the generalized AE seizures has brain stem structures as the substrate. It was shown, that hierarchical set of structures includes inferior colliculus (IC) and progresses afterwards to deep layers of superior colliculus, pontine reticular formation (PRF) and periaqueductal gray (PAG). This was shown both for AE of genetic origin and for AE induced by ethanol withdrawal in special experiments. Thus colliculi inferior and superior are the brain structures of prime interest for gene expression comparisons in different rat strains(11, 12).

2. Methods

Animals. Brain tissue (namely corpora quadrigemina) from male rats aging 4 months was taken after rapid decapitation, quickly frozen in liquid nitrogen for further analysis (KM, n=4, "0", n=4, Wistar, n=4.

KM rats were from 43th generation of inbreeding (brother-sister mating). Rat "0" were from 24^{th} generation of selection for lack of seizures in response to sound, the initial population being F2 hybrids of KM and Wistar (specially chosen for the lack of AE in three successive tests (5). Wistar rats (originated from PushchinoDepartment of Experimental Biology with Vivarium) were provided by laboratory animals firm, Biology Department MSU. Animals were maintained in plastic cages (5-7 in each) under normal conditions of lighting and temperature (22^0 C), food and water *ad lib.*

The experiments were performed in accordance with EC Declaration 2010.

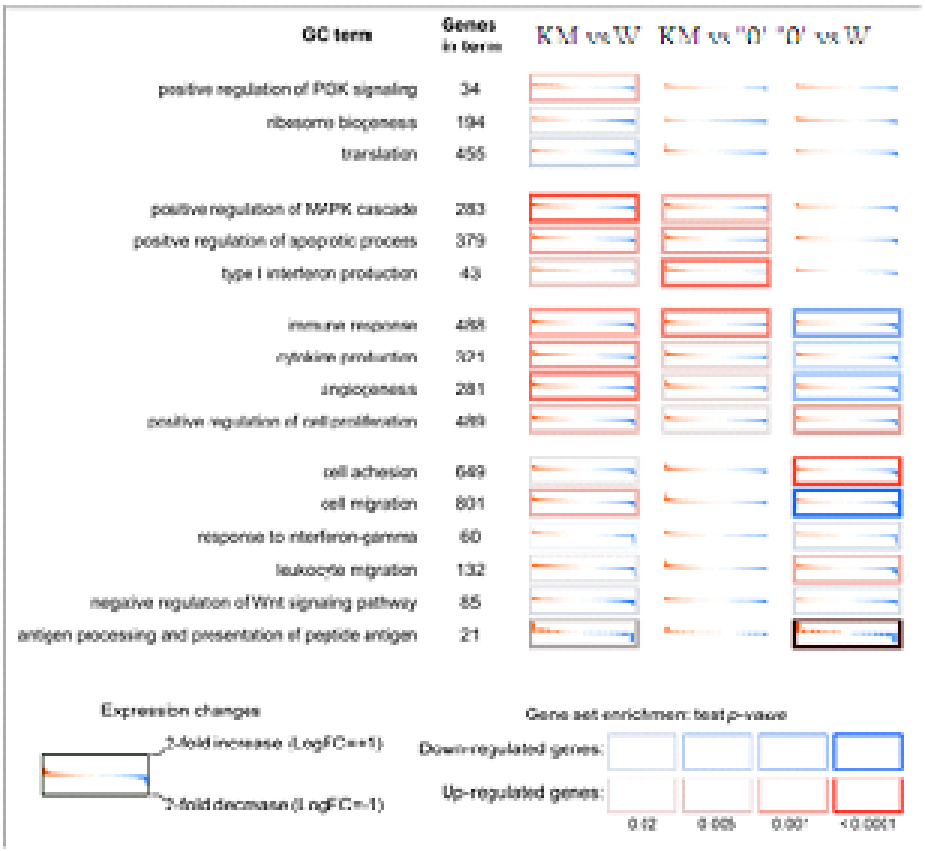

Figure 2: Gene ontology analysis of gene expression in Wistar, KM and ”0” rats (several examples).

Total RNA extraction from rat brain tissue was performed with RNAzol reagent (Molecular Research Center, USA) according to the manufacturer’s protocol. The concentration and quality of RNA was determined with a Qubit Fluorometer (Invitrogen) and Agilent BioAnalyzer 2100 (RIN>8), respectively.Libraries for RNA-seq were prepared using the NEB Next Ultra II Directional RNA Library Prep Kit for Illumina (NEB, USA) according to the manufacturer’s guidelines. The 80-bp single-end sequencing was conducted on an Illumina NextSeq 500 platform.Deep sequencing provided ~ 15-20 million reads for each library. Processing of the raw sequence data was performed using PPLine script (13), which included mapping of the reads to the reference genome with STAR(14) following adapter, length and quality trimming by Trimmomatic(15). Differential gene expression analysis was performed with the edgeR package(16). Gene Ontology and KEGG enrichment analyses were performed using the top GO (v.2.36.0) and cluster Profiler Bioconductor packages(17). Visualization of the gene set enrichment analysis (GSEA) was performed using custom scripts written in Python and R.

Synthesized from total RNA cDNA was used for ForQuantitative PCR with an MMLV RT kit **(**Evrogen, Moscow, Russia). All qRT-PCR reactions were conducted using the SYBR

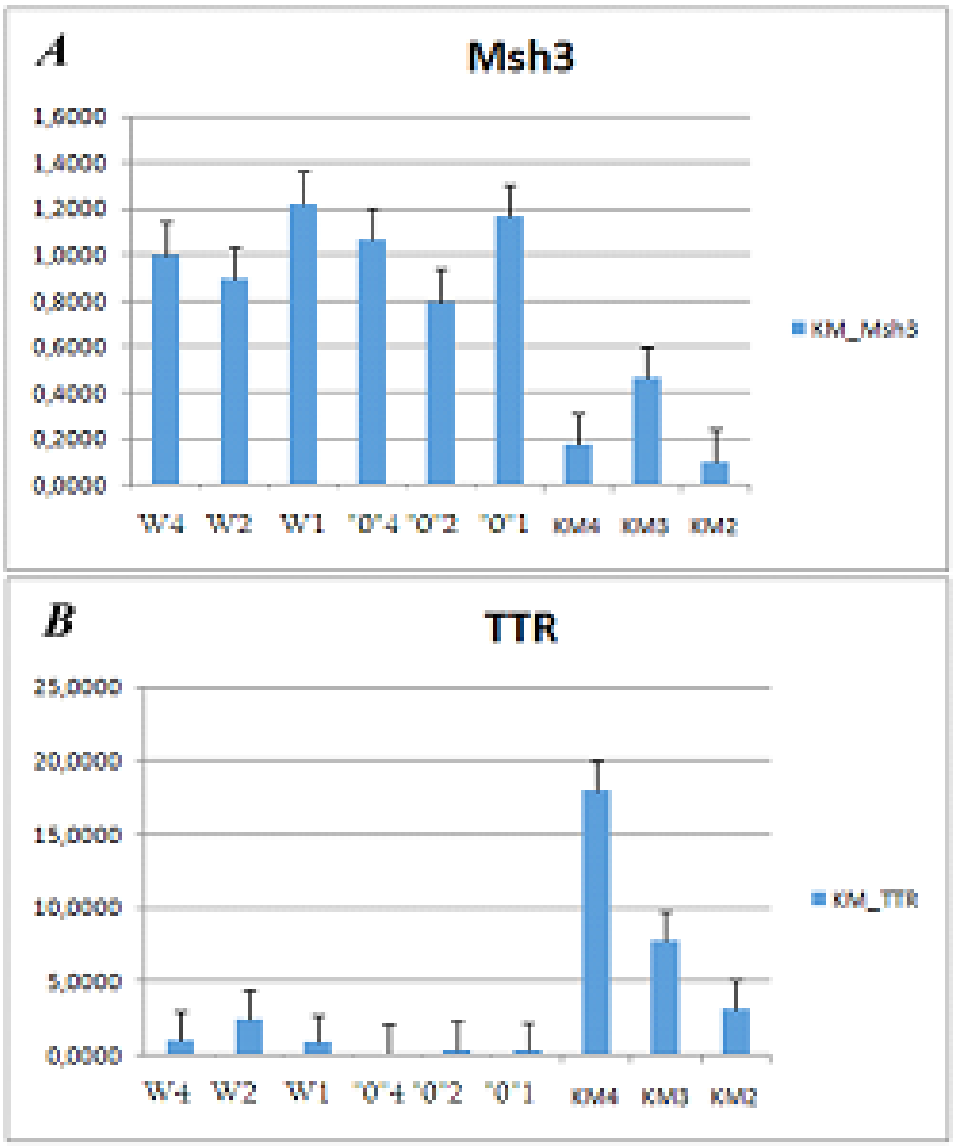

Figure 3: Expression levels of *Msh3* and *TTR* genes in Wistar, KM and "0" rats.

Green fluorescent dye (Evrogen, Russia) in an ABI PRISM VR 7500 device (Applied Biosystems). The relative expression of the studied genes was calculated based on the ΔΔCt method (18).

3. Results and Discussion.

Transcriptome analysis permitted to identify genes with expression level significantly different in the KM strain vs both non-prone groups (Wistar and "0") (figs. 2-4). Those were the genes encoding the regulatory subunits of K+ and Ca2+ voltage-gated channels - Kcne2, Kcne5 genes and Cacng4, and are also involved in seizure production in humans and in GASH AE model(19). KM strain demonstrated the higher (than in

non-prone rats) level of expression of genes involved in the positive regulation of the Ras/MAPK cascade, which is associated with the development of status epilepticus, cytokines production and immune response (20, 21).The next characteristic difference between the KM strain from Wistar and ”0” is a multiple increase in the level of expression of the TTR (transthyretin) gene, which is known to bind thyroxin and retinol (fig. 3). The increased expression of this gene (also shown in hamster AE model) could indicate that the genetic pathology in genetic AE start to express at the early brain ontogeny. The same conclusion could be driven from the fact, that practically all brain neurotransmitter systems in AE prone rats show the deviant levels both in the background and after seizure development(22). This could also signify that some early mutation event occurred rather frequently in rodent brain which affect brain development as a whole, but which is compatible with normal development and function in the absence of sound. This cautious assumption is confirmed by the finding of differences in mitochondrial function in KM rats vs Wistar as well (23). Finally, the KM strain showed the significant decrease in the expression of the Msh3 gene (fig. 3), which is responsible for the post-replicative DNA mismatch repair. Also the mutation in Msh3 was found in the GASH/Sal, the other AE model(24). It was previously shown that the 101/HY mouse strain, which is characterized by intense AE, is hypersensitive to chemical mutagens (with the mutation in the locus controlling the DNA repair) (25). Perhaps the violations in the DNA repair system could be the primary factor leading to the accumulation of mutations in other genes, which, in turn, could promote the AE and made these genotypes to be easily selected for increased AE proneness.

4. Conclusion

The transcriptome analysis revealed a number of gene expression patterns characteristic for AE proneness in rodent models. The DNA repair system defects could be the primary factor leading to the accumulation of mutations, which, in turn, promote AE.

5. Funding

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