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Invited Paper

Full-genome study of gene expression in lumbar spinal cord of mice after 30-day space flight on Bion-M1 biosatellite

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1. Introduction

Zero-gravity is one of the factors that negatively affect a man in space and it is not a surprise as the evolution of all living things proceeded in a one-G environment. The negative effects of zero-gravity set in while in space, but clinically manifest themselves following the cosmonauts' return to Earth, the usual one-G environment. All the systems of the organism, which adapted to the virtually weight-free environment, become incapable of regular performance in a one-G environment.

The effects of zero-gravity most strongly manifests in the impaired performance of the locomotor system: the strength and endurance of the muscles decrease, the structure and properties of the bones undergo transformation, and the systems responsible for constructing movements malfunction. This pathological condition is called a “Hypogravitational Locomotor Syndrome” (HLS). The history of long space flights shows that the most effective way of preserving the cosmonauts' performance and preparing them for the return to Earth is a regularly performed complex set of physical exercises in conditions imitating a one-G environment during the flight [1]. However, even diligent performance of specifically designed prophylactic sets of exercises fails to completely curb the development of HLS. It is obvious that the success of long inter-planetary flights will greatly depend on achievements in study of the pathogenesis of the HLS on the molecular, cellular and tissue levels.

Animals that have been in space in a biosatellite or held in an environment imitating zero-gravity play an important role in studies of HLS [2–4]. Such studies have shown HLS develops from an impaired afferent impulse from the limbs.

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This happens due to the fact that the parts most sensitive to mechanical stimuli do not experience the effect of footing in zero-gravity [5,6]. The studies that we have conducted on skeletal muscles, neuro-muscular synapses and spinal cord motoneurons have lead to the conclusion that demyelination of locomotor nerves, which leads to a lower speed of conducting the potentials of action in damaged nerve fibers, plays an important role in the pathogenesis of the HLS (when modeling the after effects of hypogravitation on Earth) [7]. Molecular and biological studies show that the myeliniation of long neurons deteriorates due to changes in the expression of genes coding the proteins of myelin membranes [8,9]. It is evident, however, that a mechanical extrapolation of the data acquired on Earth to the conditions of an orbital flight is unacceptable. Moreover the negative effect of space flights on human organism includes not only the condition of weightlessness. The stresses and accompanying the space flight overloading at a launch and at a landing, an artificial ecosystem demanding specific physical and psychological behavior of astronauts may have their own impact on common response of human organism to space flight.

The «Bion-M1» project allowed us to compare the data concerning the mechanism of the HLS development in the animals that were part of a space expedition and the animals with the pathology modeled on Earth. Within this project we conducted a full genome study of the lumbar spinal cord of mice that spent 30 days in space. However the analysis of the gene expression obtained using Microarray technology could not exclude the contribution to HLS the accompanying space flight factors.

2. Materials and methods

Experiments were conducted on male mice of the C57BL/6J ("Puschino" laboratory animals breeding nursery in Puschino, Moscow region). The animals were divided into two groups. The "flight" group' mice that had spent 30 days in space ($n=2$) and the "control" group' mice ($n=2$) that stayed on Earth. The spinal cord of the "flight" group mice was extracted 14 h following the biosatellite's landing. The spinal cord of the control group mice was extracted at the same time. Then the lumbar spinal cord was frozen in liquid nitrogen and kept until the beginning of the study at -80 °C. Total RNA was extracted from the spinal cord of the mice, by an RNeasy Mini Kit (Qiagen, Valencia, CA). The quality of RNA was confirmed with 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) where the 18S and 28S ribosomal bands were clearly visible and determined by electrophoresis on a 1% agarose gel and ethidium bromide staining. Gene expression profiles were determined using 500 ng of RNA in Mouse GE 4×44K v2 Microarray Kit (four arrays; Agilent Technologies). Microarray analysis procedures were conducted as per manufacturer's instructions. Quality control of the feature was performed using the settings recommended by Agilent Technologies. Signal background was subtracted and the signal intensity of each gene was globally normalized using locally weighted scatterplot smoothing. The recalculation of the gene expression and normalization was conducted using SuBio Platform software. Gene functional category analyses were performed. The number of genes corresponding to each Gene Ontology term among all genes was compared to the number among significantly regulated genes using Fisher's Exact Test. p Values of 0.05 or less were considered statistically significant.

3. Results

Out of 39486 specified genes on the microchip platform 134 genes showed a significant increase and 41 genes – a significant decrease in the expression in the material of the lumbar spine of the spinal cord of the "flight" group mice when compared to the "control" group (Table 1). The preliminary analysis of the genes that changed expression to the "flight" revealed different functional gene groups (enzymes, receptors, channels, signal proteins, etc.) as well as many predicted genes and genes with unknown function. Due to the fact that the spinal cord is a complicated cellular ensemble of many different types of cells we are unable to ascertain in which neuronal or non-neuronal cells the found genetic changes are occurring. Although, among the documented genes, we were searching for the genes responsible for the development of HLS. Based on the well-known data explaining the mechanisms of HLS development the more attention was paid to signaling in the motor neuron–skeletal muscle fiber system. It is worth to mention to the data concerning drastic changes in the expression of genes coding the proteins of calcium, potassium and sodium channels directly involved in motor neurons excitation and nerve impulse conduction by axons to skeletal muscle fibers. Changes in expression of genes encoding enzymes controlling myelination status may contribute to disorders of motor neuron function as well. Intriguing part of transcriptome examination is the detection of the genes with unknown function. Thus, the pilot analysis suggests that various functional groups of genes in different types of cells in spinal cord may be involved in the development of HLS and some changes in gene expression certainly happened in motor neurons innervating affected skeletal muscles.

4. Discussion

Previously we conducted a study on the transcriptome of the tissues of the lumbar spine of the spinal cord of mice after a 30-day antiorthostatic display of hind limbs [9].

Comparing the results of a full genome analysis of the spinal cord parts with a localization of motoneurons innervating the muscles of lower limbs (including the postural muscles that react to the hypogravitation the fastest) of the animals that were in space and the animals with the HLS modeled on Earth showed significant differences. For instance, we did not obtain confirmation of the changes expected in the expression of genes coding the myelin proteins. The data obtained gives serious reason to suppose that the pathogenesis of locomotor impairments in genuine zero-gravity and Earth-modeled zero-gravity can be significantly different. At the same time we cannot exclude the factor of the small amount of

Table 1
List of genes with up- and down regulated expression in mice after 30 days space flight.

	Gene	GenBank number	Expressed product	Cont.	Exp.	*E*/*C* (log2)	*p*-Value
	Up						
1	Nlrp4d	XM_001481310	NLR family, pyrin domain containing 4D	1.93	16.04	8.18	0.01
2	Mup20	NM_001012323	Major urinary protein20	2.29	15.32	7.13	0.03
3	A730049H05Rik	XR_035139	RIKEN cDNA A730049H05 gene	1.57	9.03	5.28	0.06
4	Tssk3	NM_080442	Testis-specific serine kinase 3	1.97	11.16	5.05	0.08
5	Zfp541	NM_001099277	Zinc finger protein541	1.60	6.87	4.22	0.02
6	Kctd8	AK082563	Potassium channel tetramerisation domain containing 8	1.68	6.79	4.05	0.00
7	Lyg2	NM_001033427	Lysozyme G-like 2	1.49	6.21	4.04	0.03
8	Btnl5	NR_004051	Butyrophilin-like 5	1.84	7.61	4.00	0.04
9	Gjb4	NM_008127	Gap junction protein, beta 4	1.85	8.19	3.99	0.10
10	Gm21498	NM_001270613	Predicted gene, 21498	1.83	7.03	3.81	0.02
11	Olfr790	NM_146933	Odorant receptor 790	2.40	8.94	3.73	0.06
12	Agbl4	AK016502	ATP/GTP binding protein-like 4	2.54	9.63	3.72	0.05
13	Col6a5	NM_001167923	Collagen, type VI, alpha 5	2.03	7.55	3.67	0.02
14	Olfr272	NM_146839	Odorant receptor 272	2.29	8.02	3.56	0.02
15	Smpd5	NM_001195537	Sphingomyelin phosphodiesterase 5	1.66	5.89	3.54	0.01
16	Il1rl2	NM_133193	Interleukin 1 receptor-like 2	1.64	5.73	3.49	0.01
17	Olfr566	NM_001011536	Odorant receptor 566	1.67	6.10	3.49	0.07
18	Prrxl1	NM_001001796	Paired coupled homeobox protein-like 1	2.04	7.18	3.48	0.03
19	Gapt	NM_177713	Grb2-binding adapter, transmembrane	1.92	6.78	3.47	0.03
20	4930565N06Rik	NR_040476	RIKEN cDNA 4930565N06 gene	1.74	5.88	3.44	0.02
21	Ttc6	XM_003084900	Tetratricopeptide repeat domain 6	3.37	11.42	3.39	0.01
22	Cym	NM_001111143	Chymosin	1.77	6.15	3.37	0.04
23	Gm10831	XR_168543	Predicted gene 10831	2.71	9.34	3.34	0.08
24	9930013L23Rik	NM_030728	RIKEN cDNA 9930013L23 gene	1.78	5.85	3.24	0.02
25	F7	NM_010172	Coagulation фактор VII	2.15	6.86	3.21	0.01
26	4921517O11Rik	AK014914	RIKEN cDNA 4921517O11 gene	1.53	4.91	3.19	0.01
27	Olfr273	NM_146824	Odorant receptor 273	1.78	5.64	3.17	0.03
28	Megf6	NM_001162977	Multiple EGF-like-domains 6	1.85	6.12	3.16	0.07
29	1700058G18Rik	NR_028107	RIKEN cDNA 1700058G18 gene	1.91	6.03	3.15	0.01
30	Vezt	DQ025533	Vezatin, adherens junctions transmembrane protein	1.68	5.30	3.11	0.03
31	9330118I20Rik	AK020366	RIKEN cDNA 9330118I20 gene	2.08	6.55	3.10	0.03
32	1600029D21Rik	NM_029639	RIKEN cDNA 1600029D21 gene	1.68	5.28	3.01	0.07
33	3110009E18Rik	NM_001172074	RIKEN cDNA 3110009E18 gene	3.39	10.19	3.00	0.02
34	Serpina5	NM_172953	Serine (or cysteine) peptidase inhibitor, clade A, member 5	1.90	5.71	2.98	0.02
35	Tespa1	NM_183264	Thymocyte expressed, positive selection coupled 1	1.74	5.15	2.96	0.01
36	Ralgapa2	AK038838	Ral GTPase activating protein, alpha subunit 2 (catalytic)	2.55	7.66	2.95	0.04
37	Gm6521	AK039704	Predicted gene 6521	2.18	6.41	2.93	0.01
38	Scn7a	NM_009135	Sodium channel, voltage-gated, type VII, alpha	1.89	5.49	2.92	0.01
39	Adamts13	NM_001001322	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 13	1.51	4.36	2.89	0.01
40	Vmn1r168	NM_001166842	Vomeronasal 1 receptor 168	4.33	12.42	2.87	0.02
41	Tmem239	NM_025753	Transmembrane 239	1.65	4.71	2.85	0.01
42	Gm21637	NM_001270685	Predicted gene, 21637	2.64	7.33	2.84	0.07
43	Serpina3k	NM_011458	Serine (or cysteine) peptidase inhibitor, clade A, member 3 K	2.48	6.73	2.83	0.08
44	Gm2447	NR_038079	Predicted gene 2447	1.73	4.99	2.83	0.05
45	Olfr815	NM_146670	Odorant receptor 815	1.56	4.39	2.80	0.01
46	Krt24	NM_029393	Keratin 24	1.95	5.44	2.80	0.01
47	Mageb3	NM_008545	Melanoma antigen, family B, 3	1.59	4.45	2.80	0.02
48	4930504O13Rik	NM_207527	RIKEN cDNA 4930504O13 gene	2.05	5.75	2.77	0.02
49	4933435G04Rik	AK017069	RIKEN cDNA 4933435G04 gene	1.88	5.27	2.75	0.04
50	Fam154a	NM_001081096	Family with sequence similarity 154, member A	1.67	4.79	2.74	0.09
51	Gm7056	NR_037571	Predicted gene 7056	3.52	9.48	2.73	0.02
52	Nsun6	NM_001165942	NOL1/NOP2/Sun domain family member 6	3.92	10.49	2.72	0.03
53	1700125G22Rik	BY707381	RIKEN cDNA 1700125G22 gene	2.78	7.43	2.72	0.07
54	Kcnmb3	NM_001195074	Potassium large conductance calcium-activated channel, subfamily M, beta member 3	2.23	6.08	2.69	0.05
55	Pebp4	NM_028560	Phosphatidylethanolamine binding protein4	1.60	4.40	2.66	0.07
56	Olfr204	NM_146992	Odorant receptor 204	1.88	5.02	2.64	0.04
57	Atp13a4	AK029367	ATPase type 13A4	1.69	4.48	2.63	0.02
58	Cyp7a1	NM_007824	Cytochrome P450, family 7, subfamily a, polypeptide 1	2.13	5.81	2.63	0.09
59	5430434I15Rik	NR_040541	RIKEN cDNA 5430434I15 gene	2.52	6.49	2.63	0.05
60	Ifih1	NM_027835	Interferon induced with helicase C domain 1	2.23	5.86	2.62	0.03
61	Fbxl13	NM_001199632	F-box and leucine-rich repeat protein13	1.66	4.35	2.61	0.00
62	Skap1	AK162116	Src family coupled phosphoprotein1	1.95	5.18	2.59	0.05
63	Ikzf4	NM_011772	IKAROS family zinc finger 4	2.17	5.64	2.58	0.03
64	Olfr1352	NM_147071	Odorant receptor 1352	2.15	5.50	2.57	0.01
65	Hsd3b6	NM_013821	Hydroxy-delta–5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 6	1.55	3.95	2.55	0.02

Table 1 (*continued*)

	Gene	GenBank number	Expressed product	Cont.	Exp.	*E/C* (log2)	*p*-Value
66	Spdya	NM_001142631	Speedy homolog A (Xenopus laevis)	2.40	6.07	2.55	0.01
67	Gm6260	NR_040405	Predicted gene 6260	2.02	5.25	2.54	0.06
68	Gm3902	XR_140984	Predicted gene 3902	2.40	6.04	2.54	0.04
69	1700120E14Rik	NR_045368	RIKEN cDNA 1700120E14 gene	2.37	5.83	2.52	0.08
70	1700123I01Rik	NM_001165919	RIKEN cDNA 1700123I01 gene	2.01	4.94	2.51	0.08
71	Iba57	NM_001270791	IBA57, iron-sulfur cluster assembly homolog (S. cerevisiae)	4.92	12.43	2.50	0.02
72	Prl2c5	NM_181852	Prolactin family 2, subfamily c, member 5	3.05	7.56	2.50	0.02
73	2610037D02Rik	NR_040423	RIKEN cDNA 2610037D02 gene	3.00	7.27	2.48	0.07
74	Gm8369	NM_001164202	Predicted gene 8369	3.28	8.09	2.46	0.05
75	Olfr899	NM_146479	Odorant receptor 899	1.79	4.52	2.46	0.07
76	Olfr1436	NM_146687	Odorant receptor 1436	1.96	4.82	2.46	0.02
77	Arhgdia	NM_133796	Rho GDP dissociation inhibitor (GDI) alpha	1.60	3.90	2.45	0.01
78	Tmpo	NM_001080134	Thymopoietin	1.77	4.34	2.44	0.04
79	Gm10277	XR_141054	Predicted gene 10277	2.16	5.22	2.42	0.02
80	Trim38	NM_001029935	Tripartite motif-containing 38	1.80	4.36	2.42	0.01
81	Olfr1097	NM_146843	Odorant receptor 1097	2.11	5.19	2.41	0.06
82	Slco1b2	NM_020495	Solute carrier organic anion transporter family, member 1b2	2.16	5.02	2.41	0.09
83	Gm9918	XR_168542	Predicted gene 9918	2.88	6.71	2.40	0.10
84	D6Ertd527e	NM_001167937	DNA segment, Chr 6, ERATO Doi 527, expressed	1.86	4.50	2.40	0.03
85	Cldn2	NM_016675	Claudin 2	1.65	3.95	2.40	0.01
86	Zim1	NM_011769	Zinc finger, imprinted 1	3.48	8.02	2.39	0.10
87	Gm4312	NM_001166636	Predicted gene 4312	2.06	4.90	2.38	0.01
88	Psg17	NM_007677	Pregnancy specific glycoprotein17	1.71	4.05	2.37	0.01
89	Gm9744	AK015109	Predicted gene 9744	3.58	8.29	2.37	0.07
90	Tmprss11g	NM_177162	Transmembrane protease, serine 11 g	2.00	4.78	2.36	0.05
91	Gm20811	XM_003085466	Predicted gene, 20811	1.82	4.29	2.35	0.02
92	Mageb4	NM_001033492	Melanoma antigen, family B, 4	1.70	4.00	2.35	0.02
93	Gm4787	NM_001038995	Predicted gene 4787	2.16	5.00	2.34	0.04
94	Plxdc1		Plexin domain containing 1	2.62	6.13	2.30	0.05
95	Spag11b	NM_001034905	Sperm coupled antigen 11B	2.86	6.59	2.29	0.02
96	Olfr1231	NM_146454	Odorant receptor 1231	2.26	5.17	2.27	0.02
97	A430054B03	AK040058	Uncharacterized proteinA430054B03	1.81	4.10	2.27	0.02
98	Cacna1h	AB041801	Calcium channel, voltage-dependent, T type, alpha 1 H subunit	2.34	5.25	2.27	0.05
99	Slc16a7	AK085398	Solute carrier family 16 (monocarboxylic acid transporters), member 7	1.96	4.40	2.24	0.00
100	Sly	NM_201530	Sycp3 like Y-linked	5.73	12.94	2.23	0.06
101	Sult6b1	NM_001163625	Sulfotransferase family, cytosolic, 6B, member 1	2.83	6.29	2.21	0.05
102	Il2rb	NM_008368	Interleukin 2 receptor, beta chain	2.83	6.17	2.19	0.06
103	Gm10752	XR_140995	Predicted gene 10752	1.63	3.56	2.18	0.04
104	Klra7	NM_014194	Killer cell lectin-like receptor, subfamily A, member 7	1.64	3.55	2.17	0.02
105	Arl13a	NM_028947	ADP-ribosylation фактор-like 13A	2.22	4.79	2.17	0.02
106	Gc	NM_008096	Group specific component	7.97	17.26	2.16	0.02
107	Ahdc1	NM_146155	AT hook, DNA binding motif, containing 1	2.18	4.69	2.15	0.00
108	Serpinb12	NM_027971	Serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 12	2.19	4.68	2.14	0.02
109	Olfr1065	NM_146408	Odorant receptor 1065	2.27	4.92	2.14	0.04
110	4930557K07Rik	CB558021	RIKEN cDNA 4930557K07 gene	2.70	5.78	2.14	0.02
111	AI314831	NR_015462	Expressed sequence AI314831	1.63	3.49	2.14	0.03
112	Ocm	NM_033039	Oncomodulin	2.38	5.13	2.13	0.03
113	Olfr630	NM_147098	Odorant receptor 630	1.48	3.13	2.12	0.03
114	Olfr222	NM_001011789	Odorant receptor 222	4.68	9.92	2.12	0.03
115	Tenm1		Teneurin transmembrane protein1	10.49	22.32	2.11	0.07
116	Vmn1r235	NM_134199	Vomeronasal 1 receptor 235	1.70	3.64	2.11	0.09
117	Gm10632	AK139634	Predicted gene 10632	3.86	8.07	2.10	0.01
118	Olfr1262	NM_146974	Odorant receptor 1262	3.27	6.85	2.10	0.06
119	Olfr878	NM_146798	Odorant receptor 878	2.05	4.35	2.09	0.06
120	Olfr1263	NM_146794	Odorant receptor 1263	2.12	4.51	2.09	0.08
121	Sftpa1	NM_023134	Surfactant coupled proteinA1	2.21	4.61	2.09	0.02
122	Uty	NM_009484	Ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	8.76	18.19	2.07	0.02
123	Shprh	NM_001077707	SNF2 histone linker PHD RING helicase	8.81	18.02	2.07	0.05
124	4930455D15Rik	NR_045381	RIKEN cDNA 4930455D15 gene	3.90	8.04	2.07	0.01
125	B230378P21Rik	NR_040277	RIKEN cDNA B230378P21 gene	2.46	5.13	2.05	0.08
126	Bdkrb1	NM_007539	Bradykinin receptor, beta 1	2.36	4.70	2.05	0.10
127	4930470F04Rik	XM_003946035	RIKEN cDNA 4930470F04 gene	2.01	4.09	2.04	0.02
128	4930478K11Rik	AK019647	RIKEN cDNA 4930478K11 gene	4.59	9.37	2.03	0.02
129	9630025H16Rik	AK079329	RIKEN cDNA 9630025H16 gene	2.10	4.28	2.03	0.09
130	Olfr808	NM_146928	Odorant receptor 808	1.81	3.67	2.03	0.03
131	Abca13	NM_178259	ATP-binding cassette, subfamily A (ABC1), member 13	3.53	7.08	2.02	0.03

Table 1 (*continued*)

	Gene	GenBank number	Expressed product	Cont.	Exp.	*E*/*C* (log2)	*p*-Value
132	C87882	BG067895	Expressed sequence C87882	1.45	2.92	2.02	0.01
133	Trim42	NM_030219	Tripartite motif-containing 42	5.25	10.68	2.01	0.06
134	Prss22	NM_133731	Protease, serine, 22	1.87	3.75	2.01	0.01
	Down						
1	Myot	NM_001033621	Myotilin	41.09	1.84	0.05	0.02
2	Ttn	NM_011652	Titin	14.87	1.30	0.09	0.01
3	Igkv2-112	M19909	Immunoglobulin kappa variable 2-112	11.73	1.68	0.14	0.00
4	Flcn		Folliculin	6.50	1.86	0.30	0.10
5	Pbk	NM_023209	PDZ binding kinase	18.03	5.50	0.31	0.03
6	Gm11437	NM_001037932	Predicted gene 11437	4.84	1.51	0.33	0.07
7	Fitm1	NM_026808	Fat storage-inducing transmembrane protein1	4.56	1.48	0.33	0.03
8	Gm10757	XR_140526	Predicted gene 10757	4.42	1.44	0.33	0.03
9	Uts2	NM_011910	Urotensin 2	4868.00	1630.45	0.33	0.00
10	4930448F12Rik	NR_046032	RIKEN cDNA 4930448F12 gene	4.21	1.38	0.34	0.04
11	Gm1140	NM_001126317	Predicted gene 1140	4.49	1.53	0.34	0.01
12	Tmem150b	NM_001142792	Transmembrane protein 150B	5.09	1.78	0.35	0.02
13	Gm362	NM_001195271	Predicted gene 362	5.12	1.79	0.35	0.06
14	Rdh18-ps	NR_037604	Retinol dehydrogenase 18, pseudogene	4.73	1.69	0.35	0.03
15	Gm9999	NR_033461	Predicted gene 9999	11.38	4.02	0.37	0.06
16	Vmn2r103	NM_001104565	Vomeronasal 2, receptor 103	5.37	1.97	0.37	0.01
17	Myh8	NM_177369	Myosin, heavy polypeptide 8, skeletal muscle, perinatal	4.45	1.66	0.37	0.01
18	H1fnt	NM_027304	H1 histone family, member N, testis-specific	4.76	1.84	0.39	0.00
19	AW050000	AW050000	Expressed sequence AW050000	5.71	2.27	0.39	0.05
20	Vmn2r110	NM_001104572	Vomeronasal 2, receptor 110	3.70	1.47	0.40	0.01
21	Kcnmb1	NM_031169	Potassium large conductance calcium-activated channel, sub-family M, beta member 1	9.86	3.95	0.40	0.05
22	Serpina4-ps1	BC031891	Serine (or cysteine) peptidase inhibitor, clade A, member 4, pseudogene 1	4.05	1.56	0.40	0.09
23	Tspan18	AK032765	Tetraspanin 18	4.54	1.84	0.41	0.06
24	Olfr781	NM_146728	Olfactory receptor 781	4.44	1.85	0.42	0.00
25	1700009N14Rik	NM_001081095	RIKEN cDNA 1700009N14 gene	8.84	3.67	0.43	0.06
26	AF067061	NM_199060	cDNA sequence AF067061	5.47	2.45	0.43	0.10
27	Fam50b	NM_138746	Family with sequence similarity 50, member B	3.62	1.56	0.43	0.03
28	Cacng1	NM_007582	Calcium channel, voltage-dependent, gamma subunit 1	4.51	2.00	0.45	0.03
29	Gm13083	NM_001126324	Predicted gene 13083	3.35	1.46	0.45	0.10
30	Samd3	NM_001013766	Sterile alpha motif domain containing 3	3.07	1.42	0.47	0.04
31	Dsg3	NM_030596	Desmoglein 3	3.90	1.81	0.47	0.06
32	Olfr616	NM_147099	Olfactory receptor 616	10.27	4.87	0.47	0.01
33	Cdkl5	NM_001024624	Cyclin-dependent kinase-like 5	25.92	12.49	0.48	0.04
34	Crlf1	NM_018827	Cytokine receptor-like factor 1	4.25	2.04	0.48	0.04
35	Metrnl	NM_144797	Meteorin, glial cell differentiation regulator-like	2.89	1.40	0.49	0.02
36	Ptprq	NM_001081432	Protein tyrosine phosphatase, receptor type, Q	4.00	1.92	0.49	0.06
37	Il1rl1	NM_001025602	Interleukin 1 receptor-like 1	4.01	1.95	0.49	0.03
38	Hyal6	NM_028920	Hyaluronoglucosaminidase 6	15.19	7.47	0.49	0.02
39	Gm4981	NM_001034869	Predicted gene 4981	6.88	3.38	0.49	0.01
40	A930006I01Rik	NR_040332	RIKEN cDNA A930006I01 gene	15.56	7.55	0.49	0.09
41	1700025C18Rik	NR_033448	RIKEN cDNA 1700025C18 gene	8.25	4.11	0.50	0.02

«space material» (due to its uniqueness), which could be the reason for the differences in the results of the «modeled» and «flight» studies. In spite of the intriguing results the compassion of these two studies in terms of HLS development is speculative mostly because of space flight is a multifactorial condition that involves many other aspects than microgravity. It is obvious that the main pathogenic factor in development of HLS is microgravity and the main affected organ is postural skeletal muscles. Earlier we hypothesized that the one of the key factors in HLS pathogenesis are spinal motoneurons. Some changes in gene expression in spinal cord documented in this research are responsible for HLS, but others may be due, for example, to the overloading and stress during the landing. The list of upregulated and downregulated genes presented here is mostly the resource for the discovery of the real mechanism for HLS development.

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References

[1] I.B. Kozlovskaia, I.D. Pestov, A.D. Egorov, The countermeasure system for extended space flights, Aviakosm Ekol. Med. 42 (2008) 66–73.

[2] A.I. Grigor'ev, E.A. Il'in, Animals in space, Vestnik Dokl. Acad. Sci. 77 (11) (2007) 963–986.
[3] E.R. Morey-Holton, R.K. Globus, Hindlimb unloading rodent model: technical aspects, J. Appl. Physiol. 92 (4) (2002) 1367–1377.
[4] E.N. Lipets, E.V. Ponomareva, I.V. Ogneva, I.M. Vikhliantsev E.V. Karaduleva, N.L. Kratashkina, S.L. Kuznetsov, Z.A. Podlubnaia B.S. Shenkman, Contractile properties of fibers and cytoskeletal proteins of gerbil's hindlimb muscles after space flight, Aviakosm Ekol. Med. 43 (3) (2009) 34–39.
[5] A.I. Grigor'ev, I.B. Kozlovskaia, B.S. Shenkman, The role of support afferents in organisation of the tonic muscle system, Ross. Fiziol. Zh. Im I. M. Sechenova 90 (5) (2004) 508–521.
[6] A.I. Grigor'ev, B.S. Shenkman, Skeletal muscle in the unsupported world. Another application of physiology, signal system, Vestnik Dokl. Acad. Sci. 78 (4) (2008) 337–345.
[7] R.R. Islamov, N.I. Lannik, G.F. Shaimardanova, P.N. Rezvyakov O.V. Tyapkina, A.A. Rizvanov, Y.A. Chelyshev, I.B. Kozlovskaya E.E. Nikolskii, Effect of hindlimb unloading on myelinated fibers in the mouse lumbar spinal cord, Dokl. Biol. Sci. 452 (2013) 266–268.
[8] O.V. Tiapkina, L.F. Nurullin, P.N. Rezviakov, I.B. Kozlovskaia E.E. Nikol'skiĭ, R.R. Islamov, Myelination disorders in mechanism of hypogravity motor syndrome development, Biofizika 57 (5) (2012) 876–879.
[9] R.R. Islamov, A.A. Rizvanov, O.V. Tyapkina, B.S. Shenkman I.B. Kozlovskaya, E.E. Nikolskiy, A.I. Grigoryev, Genomic study of gene expression in the mouse lumbar spinal cord under the conditions of simulated microgravity, Dokl. Biol. Sci. 439 (2011) 197–200.