Training-Induced Gene Expression Plasticity in Cardiac Function and Neural Regulation for Ultra-Trail Runners

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Abstract

This study aims to assess the gene regulatory response from a group of 16 athletes and to observe the plasticity induced by their training regime on the gene expression response after their participation in an 82km race. Blood samples for differential gene expression (DGE) were collected before and after this effort from two groups of runners with different training regimes: elite and active. Analyses only focused on genes annotated as related to cardiac function (CF) and neural regulation (NR) from the KEGG PATHWAY Database. Thus, 13 pathways were considered accounting for a total of 629 genes.

Training regime modulated the response to exercise based on a list of 18 ranked genes with significant DGE for elite runners while remained statistically insignificant for active athletes. UQCR11, COX7C and COX4I1 genes, related to mitochondrial respiratory chain, were down-regulated which may indicate mitochondrial function impairment in cardiac muscle. Increased expression levels were obtained for PIK3R2, PLCG2, IRAK3 genes from the positive signaling cascades of neurotrophins pathway, which may reveal an improved heart rate control thanks to a better cardiac sympathetic innervation.

1. Introduction

Ultra-trail races are an ideal sport to investigate a physiological response to an endurance exercise given its significant intensity and prolonged duration [1]. The study of heart rate variability is a powerful and an extended method for better understanding the cardiac function and its regulation through the autonomic nervous system [2]. Most experiments have focused on examining the effects of exercise training versus sedentary subjects [3] or in previously sedentary individuals [4]. The training intensity, duration and type have already been related with specific genetic regulation activity [5].

The aim of this work is to analyze the gene expression plasticity, induced by distinct training regimes followed by runners, in the physiological response to an ultra-trail participation.

2. Materials and methods

2.1. Experimental design

A total of 16 runners were recruited and classified into two different training regime groups based on their weekly exercise hours: (i) Active group from three to 10 hours and (ii) Elite group: more than 10 hours.

Eight male and two female formed the active group with mean age of 38.7±4.7 years, while three male and three female belonged to the elite group (38.0±1.4 years). Characteristics of the runners are summarized in Table 1.

All individuals were experienced athletes who volunteered to participate in this experiment, which was deployed on the Cavalls del Vent 4th edition from 2012 (Cadí-Moixeró Natural Park, Catalonia, Spain). The crossing took place at altitudes of between 900 meters to 2510 meters with a total positive slope of 5200 meters approximately. Ultra-trail distance was 82 km. The active group could run a mean distance of 37.3±14.1 km and the elite group 60.3±23.9 km. Adverse weather conditions caused that only three out of 16 participants were able to complete the race.

Table 1. Runner characteristics participating in the experiment.

Id	Training level	Gender	Dist[1] [km]	Age [Years]
1[2]	Elite	Female	82	36
15	Elite	Female	82	38
2	Elite	Male	42	40
3	Elite	Female	82	39
7	Elite	Male	33	38
9[2]	Elite	Male	41	37
163	Active	Male	50	45
164	Active	Male	50	42
197	Active	Male	25	37
256	Active	Male	50	37
577	Active	Male	25	42
735	Active	Male	50	40
775[2]	Active	Male	28	28
805	Active	Male	50	36
838[2]	Active	Female	31	42
957	Active	Female	14	38

[1]Dist: Distance completed by the runner.
[2]Only pre-race expression levels are available.

2.2. Blood sampling, RNA isolation and transcriptome analysis

Venous blood samples were drawn from the antecubital vein at rest in a sitting position and collected into PAXgene Blood RNA Tubes according to the manufacturer's protocol (PreAnalytiX GmbH/QIAGEN, Switzerland/US). Samples were obtained from each subject in previous periods before the race and after completing their participation in the ultra-trail. They were stored at -80ºC and properly identified until their assay in Hospital de la Santa Creu I Sant Pau (HSCiSP) (Barcelona, Spain).

Total RNA was isolated using the PAXgene Blood RNA kit (PreAnalytiX GmbH/QIAGEN, Switzerland/US). The concentration of the extracted RNA was measured spectrophotometrically (Nanodrop 1000/ Thermo Fisher Scientific, Wilmington, US). Expression levels were randomly checked by real time PCR performed on IL-1β and CD141 genes.

For expression profiling, RNA was amplified and biotinylated using the Ambion TotalPrep RNA Amplification kit (Life Technologies, Carlsbad US) in order to be measured through hybridization onto HuGene2.0st microarrays (Affymetrix Inc., California US). HSCiSP laboratory carried out the hybridization, washing, staining, scanning and grid alignment processes in order to generate the corresponding CEL files.

2.3. Bioinformatic data analysis

A selection of biomarkers candidates to regulate and control the cardiovascular system was made based on a prior selection of related biological pathways from KEGG PATHWAY database [6]. With this purpose, a total of 13 pathways (see Table 2) belonging to circulatory system (CS) and nervous system (NS), from organismal systems category, were chosen.

Table 2. List of pathways related to CF and NR from KEGG PATHWAYS Database – circulatory system (CS) and nervous system (NS) category.

KEGG Pathway id	Pathway name
hsa04260	Cardiac muscle contraction (CS)
hsa04261	Adrenergic signaling in cardiomyocytes (CS)
hsa04270	Vascular smooth muscle contraction (CS)
hsa04720	Long-term potentiation (NS)
hsa04721	Synaptic vesicle cycle (NS)
hsa04722	Neurotrophin signaling pathway (NS)
hsa04723	Retrograde endocannabinoid signaling (NS)
hsa04724	Glutamatergic synapse (NS)
hsa04725	Cholinergic synapse (NS)
hsa04726	Serotonergic synapse (NS)
hsa04727	GABAergic synapse (NS)
hsa04728	Dopaminergic synapse (NS)
hsa04730	Long-term depression (NS)

Available CEL files (28 in total) were analyzed with the R Software for Statistical Computing (v3.2.0) [7] and BioConductor v3.1 [8]. Raw fluorescence intensity values were background corrected, quantile normalized and summarized with Robust Multichip Average through package *oligo* v1.32.0 [9]. An *ExpressionSet* object was obtained with expression level values for 53,617 transcript clusters per each athlete in the study. Quality control was performed over this object in order to detect possible outliers. A list of 629 unique biomarkers was selected based on the former pathway selection. Only this subset was considered for further assessment.

A non-supervised filtering based on overall intensity and variability was applied to discard non-informative genes resulting in 77 genes. Based on the design of the experiment, three parameters (Gender: *G*, Training level: *T* and time – pre or post-race: *PP*) were identified to have an impact on gene expression. Thus, a linear regression model was fit to each biomarker expression value (g_k) by controlling for those variables and an interaction factor between the latter as indicated below (1). Package *limma*

Table 3. Ranked genes with differential expression levels in elite vs active runners as a response to ultra-trail participation sorted by obtained adj. p-value (FDR).

Transcript	Gene	Regulation	adj. p-val	logFC	KEGG Pathways Id
16827366	ATP6V0D1	Up	2.52E-06	1.05	hsa04721
16690566	SORT1	Up	1.26E-05	1.42	hsa04722
16821614	COX4I1	Down	4.80E-05	-0.89	hsa04260
16859763	PIK3R2	Up	1.66E-04	0.86	hsa04261;hsa04722;hsa04725
16668286	GNAI3	Up	2.04E-04	0.86	hsa04261;hsa04723;hsa04724;hsa04725;hsa04726; hsa04727;hsa04728;hsa04730
16753670	IRAK3	Up	3.26E-04	1.35	hsa04722
16837391	KCNJ2	Up	4.13E-04	1.13	hsa04725
17007543	ITPR3	Down	5.62E-04	-0.82	hsa04270;hsa04720;hsa04723;hsa04724;hsa04725; hsa04726;hsa04728;hsa04730
17095111	GNAQ	Up	6.02E-04	0.77	hsa04261;hsa04270;hsa04720;hsa04723;hsa04724; hsa04725;hsa04726;hsa04728;hsa04730
16944096	ATP6V1A	Up	1.07E-03	0.83	hsa04721
16866718	UQCR11	Down	1.40E-03	-0.61	hsa04260
16821326	PLCG2	Up	1.77E-03	0.83	hsa04722
16986983	COX7C	Down	2.65E-03	-0.87	hsa04260
17002846	DUSP1	Up	5.12E-03	0.73	hsa04726
16866974	GNG7	Up	8.42E-03	0.64	hsa04723;hsa04724;hsa04725;hsa04726;hsa04727; hsa04728
16840876	VAMP2	Up	1.91E-02	0.41	hsa04721
16713085	KIF5B	Down	4.93E-02	-0.59	hsa04728
17070634	RIPK2	Down	4.98E-02	-0.53	hsa04722

v3.24.10 [10] was used for this purpose.

$$g_k \approx \beta_0 + \beta_1 G + \beta_2 T + \beta_3 PP + \beta_4 (T \cdot PP) + \epsilon \quad (1)$$

A specific contrast matrix was defined to get the relevant expression differences. Given this, estimated coefficients and standard errors were computed from the original model (1). Moderated t-statistics for these contrasts were obtained using an empirical Bayes method. Genes with significant differential expression were selected and ranked based on their resulting adjusted p-value (5%). FDR correction for multiple testing was applied. STRING database [11] was used to visualize the protein interactions based on the ranked genes.

3. Results and discussion

56 genes were found to be differentially expressed as a response to the acute physical exercise. Among them, a subset of 18 genes were only significant for elite runners group while remain insignificant for active ones, which would suggest that training regime induced a modulation effect in such response. Table 3 shows this list of genes including the KEGG pathways to which they belong. Figure 1 shows an interaction network between the proteins encoded by this subset.

A significant down-regulation is observed in UQCR11, COX7C and COX4I1 gene expression levels taken from cardiac muscle contraction pathway. They are related to mitochondrial respiratory chain function (complexes III and IV specifically). This may indicate mitochondrial impairment due to exercise overload as found in [12] during an experiment with rat myocardium. Further evidence also associates complex IV activity with muscle oxidative capacity for human skeletal muscle [13], in correspondence with our results.

Down-regulation was found for ITPR3 gene, which encodes a receptor for a second messenger that mediates the release of intracellular calcium (Ca^{2+}), a key regulator of mitochondrial function. It may reveal an improved muscle damage prevention by avoiding mitochondrial matrix Ca^{2+} overload, which leads to higher generation rate of reactive oxygen species (ROS) [14].

Neurotrophin intracellular signaling cascades were affected based on up-regulation of IRAK3, PLCG2 and PIK3R2 genes which signals enhance cell survival [3]. A previous study related neurotrophins to stability of cardiac sympathetic innervation and in consequence, an indicator of better heart rate control [15,16].

4. Conclusions

Gene expression plasticity due to a specific training regime has been analyzed in terms of cardiac function and neural regulation. A modulated biological response to physical effort has been detected identifying genes related to mitochondrial respiratory chain function and

neurotrophin signaling pathway. These results generate interesting hypothesis on future experiments with a larger population.

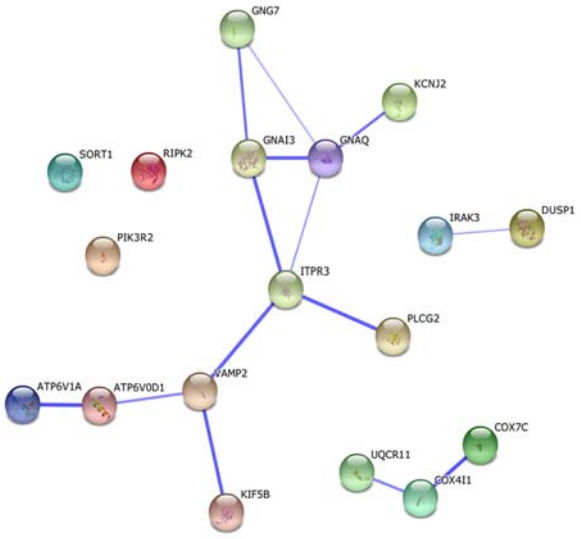

Figure 1. Protein interactions between the 18 ranked genes from Table 3 obtained by STRING database [11]. Thicker lines represent stronger associations under heterogeneous evidence codes.

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