rs1769793 variant reduces *EGLN1* expression in skeletal muscle and hippocampus and contributes to high aerobic capacity in hypoxia

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Evidence shows that *EGLN1* could control the hypoxia-inducible factor-α (HIF-1α) level by suppressing its transcriptional activity, which, in turn, regulates the cellular hypoxic response (1–3). Brutsaert et al. (4) analyze 429 Peruvian Quechua individuals and 94 US lowland referents. They identify five *EGLN1* variants (rs1769793, rs2064766, rs2437150, rs2491403, and rs479200) to be associated with higher VO_2max in hypoxia (4). They further demonstrate the role of natural selection in increasing the frequency of *EGLN1* genetic variants at high altitude (4).

However, it remains unclear how these variants affect high aerobic capacity in hypoxia, as they are located in noncoding regions of *EGLN1* or outside the *EGLN1* gene boundaries (4). In discussion, Brutsaert et al. (4) hypothesize that these variants may be regulatory, which needs to be further investigated. However, they do not directly evaluate the regulatory relation, which prompts us to conduct further investigation. Our recent expression quantitative trait loci (eQTLs) analysis indicated that genetic variants, especially in noncoding regions, could regulate gene expression (5–9). Using SNiPA**v3.3** (a tool for annotating and browsing genetic variants), we found that all five of these *EGLN1* variants were in high linkage disequilibrium with each other. Hence, we select the most significant variant rs1769793 to evaluate its association with *EGLN1* gene expression.

The eQTLs dataset is from the Genotype-Tissue Expression project (version 8) including 49 tissues (number of samples with genotype ≥ 70) (10). Here, we select the FastQTL to perform the eQTLs analysis by adjusting for some key covariates (10). The statistical significance for eQTLs analysis is a Bonferroni-corrected threshold of $P < 0.05/49 = 1.00\text{E-}03$. Meanwhile, we conduct a gene expression analysis to investigate the distribution of *EGLN1* expression in these 49 tissues. The gene expression level is quantified by transcripts per million (TPM) based on the GENCODE (encyclopedia of DNA elements) 26 annotation, collapsed to a single transcript model for each gene using a custom isoform collapsing procedure (10). Here, we select the *t* test or analysis of variance method to evaluate the distribution difference of *EGLN1* expression in different tissues. The statistical significance is $P < 0.05$.

The eQTLs analysis indicates that the rs1769793 T allele, which is associated with higher VO_2max in hypoxia, could significantly reduce *EGLN1* expression in skeletal muscle ($P = 5.20\text{E-}05$) and hippocampus ($P = 2.90\text{E-}04$). Meanwhile, the rs1769793 T allele is also associated with reduced *EGLN1* expression in other brain tissues including cortex, hypothalamus, amygdala, cerebellum, and putamen, as provided in Table 1. The gene expression analysis shows that *EGLN1* has the highest expression in skeletal muscle compared with the other 48 tissues ($P < 0.05$), as provided in Table 1.

Taken together, our findings show that *EGLN1* is mainly expressed in skeletal muscles, and rs1769793 T allele reduces *EGLN1* expression in skeletal muscle and hippocampus, which may, in turn, promote HIF-1α transcriptional activity and contribute to high aerobic capacity in hypoxia. Importantly, our results are consistent with previous findings from William G. Kaelin Jr. and colleagues (2). They revealed that inhibiting Egln1 in skeletal muscles could protect mice against myocardial ischemia–reperfusion injury (2). Hence, our findings may provide important information about the role of rs1769793 in hypoxia.

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Table 1. rs1769793 variant T allele and *EGLN1* expression in 49 human tissues

SNP	Beta	SE	*P* value	Tissue	Samples	*EGLN1* expression
rs1769793	−0.033	0.030	2.60E-01	Adipose - subcutaneous	581	40.15
rs1769793	−0.088	0.038	2.30E-02	Adipose - visceral	469	24.81
rs1769793	0.130	0.059	3.00E-02	Adrenal gland	233	21.13
rs1769793	−0.094	0.045	3.30E-02	Artery - aorta	387	27.97
rs1769793	−0.140	0.067	4.10E-02	Artery - coronary	213	27.09
rs1769793	−0.019	0.031	5.40E-01	Artery - tibial	584	34.18
rs1769793	−0.190	0.083	2.40E-02	Brain - amygdala	129	16.39
rs1769793	−0.079	0.061	2.00E-01	Brain - anterior cingulate cortex	147	17.84
rs1769793	−0.092	0.048	5.50E-02	Brain - caudate	194	18.57
rs1769793	−0.083	0.044	5.70E-02	Brain - cerebellar hemisphere	175	29.55
rs1769793	−0.089	0.042	4.00E-02	Brain - cerebellum	209	24.69
rs1769793	−0.100	0.036	5.40E-03	Brain - cortex	205	16.69
rs1769793	−0.047	0.043	2.80E-01	Brain - frontal cortex	175	19.97
rs1769793	−0.180	0.049	2.90E-04	Brain - hippocampus	165	16.89
rs1769793	−0.130	0.050	1.10E-02	Brain - hypothalamus	170	17.92
rs1769793	−0.077	0.045	1.00E-01	Brain - nucleus accumbens	202	20.62
rs1769793	−0.110	0.055	4.80E-02	Brain - putamen	170	15.60
rs1769793	−0.075	0.106	4.80E-01	Brain - spinal cord	126	23.47
rs1769793	−0.070	0.076	3.60E-01	Brain - substantia nigra	114	17.56
rs1769793	−0.024	0.038	5.20E-01	Breast - mammary tissue	396	27.40
rs1769793	0.017	0.028	5.50E-01	Cells - cultured fibroblasts	483	26.48
rs1769793	−0.044	0.072	5.40E-01	Cells - EBV-transformed lymphocytes	147	18.79
rs1769793	−0.058	0.048	2.50E-01	Colon - sigmoid	318	30.26
rs1769793	−0.015	0.030	6.20E-01	Colon - transverse	368	21.15
rs1769793	−0.056	0.047	2.40E-01	Esophagus - gastroesophageal junction	330	26.50
rs1769793	−0.021	0.027	4.40E-01	Esophagus - mucosa	497	17.00
rs1769793	−0.007	0.035	8.30E-01	Esophagus - muscularis	465	27.96
rs1769793	0.008	0.034	8.10E-01	Heart - atrial appendage	372	19.72
rs1769793	−0.002	0.029	9.30E-01	Heart - left ventricle	386	19.43
rs1769793	0.050	0.114	6.60E-01	Kidney - cortex	73	12.59
rs1769793	−0.052	0.043	2.10E-01	Liver	208	13.97
rs1769793	−0.054	0.032	9.90E-02	Lung	515	15.28
rs1769793	0.004	0.077	9.60E-01	Minor salivary gland	144	16.50
rs1769793	−0.077	0.019	5.20E-05	Muscle - skeletal	706	155.90
rs1769793	−0.049	0.038	2.10E-01	Nerve - tibial	532	36.21
rs1769793	−0.091	0.083	2.70E-01	Ovary	167	29.63
rs1769793	0.056	0.043	1.90E-01	Pancreas	305	11.36
rs1769793	0.045	0.056	4.20E-01	Pituitary	237	19.80
rs1769793	0.110	0.050	2.90E-02	Prostate	221	16.67
rs1769793	−0.036	0.030	2.30E-01	Skin - not sun exposed	517	27.21
rs1769793	−0.014	0.029	6.30E-01	Skin - sun exposed	605	30.22
rs1769793	0.001	0.042	9.90E-01	Small intestine - terminal ileum	174	17.31
rs1769793	0.036	0.051	4.90E-01	Spleen	227	17.49
rs1769793	−0.033	0.030	2.90E-01	Stomach	324	11.67
rs1769793	0.019	0.023	4.20E-01	Testis	322	14.29
rs1769793	−0.007	0.037	8.60E-01	Thyroid	574	19.08
rs1769793	−0.095	0.086	2.70E-01	Uterus	129	29.75
rs1769793	−0.017	0.100	8.60E-01	Vagina	141	25.67
rs1769793	−0.021	0.015	1.60E-01	Whole blood	670	16.48

SNP, single-nucleotide polymorphism; EBV, Epstein–Barr virus; Beta is the regression coefficient based on the effect allele. Beta > 0 and Beta < 0 means that this effect allele increases and reduces gene expression, respectively. The threshold of statistical significance for eQTLs analysis is $P < 0.05/49 = 1.00E-03$. The gene expression values are shown in TPM. The gene expression level is quantified by TPM based on the GENCODE 26 annotation, collapsed to a single transcript model for each gene using a custom isoform collapsing procedure.

1 K. K. To, L. E. Huang, Suppression of hypoxia-inducible factor 1alpha (HIF-1alpha) transcriptional activity by the HIF prolyl hydroxylase EGLN1. *J. Biol. Chem.* **280**, 38102–38107 (2005).

2 B. A. Olenchock *et al.*, EGLN1 inhibition and rerouting of α-ketoglutarate suffice for remote ischemic protection. *Cell* **164**, 884–895 (2016).

3 M. Ivan, W. G. Kaelin Jr., The EGLN-HIF O_2-sensing system: Multiple inputs and feedbacks. *Mol. Cell* **66**, 772–779 (2017).

4 T. D. Brutsaert *et al.*, Association of *EGLN1* gene with high aerobic capacity of Peruvian Quechua at high altitude. *Proc. Natl. Acad. Sci. U.S.A.***116**, 24006–24011 (2019). Correction in: *Proc. Natl. Acad. Sci. U.S.A.* **117**, 3339 (2020).

5 G. Liu, H. Zhang, B. Liu, X. Ji, Rs2293871 regulates HTRA1 expression and affects cerebral small vessel stroke and Alzheimer's disease. *Brain* **142**, e61 (2019).

6 G. Liu *et al*., rs4147929 variant minor allele increases ABCA7 gene expression and ABCA7 shows increased gene expression in Alzheimer's disease patients compared with controls. *Acta Neuropathol.* **139**, 937–940 (2020).
7 G. Liu, Y. Hu, Z. Han, S. Jin, Q. Jiang, Genetic variant rs17185536 regulates *SIM1* gene expression in human brain hypothalamus. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 3347–3348 (2019).
8 Y. Hu, S. Jin, L. Cheng, G. Liu, Q. Jiang, Autoimmune disease variants regulate *GSDMB* gene expression in human immune cells and whole blood. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E7860–E7862 (2017).
9 G. Liu, S. Jin, Y. Hu, Q. Jiang, Disease status affects the association between rs4813620 and the expression of Alzheimer's disease susceptibility gene *TRIB3*. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E10519–E10520 (2018).
10 A. Battle, C. D. Brown, B. E. Engelhardt, S. B. Montgomery; GTEx Consortium; Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group; Statistical Methods groups—Analysis Working Group; Enhancing GTEx (eGTEx) groups; NIH Common Fund; NIH/NCI; NIH/NHGRI; NIH/NIMH; NIH/NIDA; Biospecimen Collection Source Site—NDRI; Biospecimen Collection Source Site—RPCI; Biospecimen Core Resource—VARI; Brain Bank Repository—University of Miami Brain Endowment Bank; Leidos Biomedical—Project Management; ELSI Study; Genome Browser Data Integration &Visualization—EBI; Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz; Lead analysts; Laboratory, Data Analysis &Coordinating Center (LDACC); NIH program management; Biospecimen collection; Pathology; eQTL manuscript working group, Genetic effects on gene expression across human tissues. *Nature* **550**, 204–213 (2017).