A High-Dimensional Mediation Analysis Integrating Genomics and Epigenomics to Understand Adaptive Advantages and Health Risks of Chronic Hypoxia in andean Highlanders

Sacco Davide[1], De Marco Cesare[1], Calzari Lucian[2], Villafuerte Francisco[3], Zanotti Lucia[4], Parati Gianfranco[4,5], Bilo Grzegorz[4,5], Gentilini Davide[1,2]

(1) Department of Brain and Behavioral Sciences, Università di Pavia, 27100 Pavia, Italy
(2) Bioinformatics and Statistical Genomics Unit, Istituto Auxologico Italiano IRCCS, 20095 Cusano Milanino, Italy 3 Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru
(3) Department of Cardiology, Istituto Auxologico Italiano, IRCCS, Milan, Italy
(4) Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

CORRESPONDING AUTHOR: *Sacco Davide, davide.sacco02@universitadipavia.it*

This study is part of the HIGHCARE LAPS project (HIGH Altitude Cardiovascular Research – Latin American Population Study), which investigates the biological impact of chronic hypoxia in high- altitude populations, focusing on Andean communities in Peru. These highlanders have developed distinctive genetic, physiological and lifestyle adaptations that support survival in low-oxygen environments and may reduce the prevalence of certain diseases. At the same time, some health conditions might be overlooked, such as hypertension, which could be underestimated due to altered blood pressure regulation at high altitude [1,2].In this cohort, genomic analyses revealed only minor differences between individuals living at sea level in Lima and those residing above 4000 meters in Cerro de Pasco, while analyses of DNA methylation, a well-established marker of epigenetic regulation, identified several differences, particularly at CpG sites involved in adrenergic signaling in cardiomyocytes, suggesting a potential epigenetic contribution to high-altitude adaptation. However, it remains unclear whether DNA methylation actively shapes phenotypic peculiarities associated with high-altitude adaptation, such as enhanced oxygen transport, or contributes to health risks related to chronic hypoxia.

AIM

The study investigates whether epigenetic regulation, assessed through genome-wide DNA methylation, mediates phenotypic effects of chronic hypoxia in a Peruvian cohort of 96 highlanders (above 4000 meters) and 96 lowlanders (at sea level). The focus is on traits including hemoglobin, hematocrit, oxygen saturation, 24-hour systolic and diastolic blood pressure, 24-hour heart rate, respiratory rate, and hypertension risk, accounting for genetic background and various clinical and environmental confounders.

METHODS

DNA methylation and genotyping data were obtained from whole blood using the Illumina MethylationEPIC v1.0 array (866000 CpGs) and the Illumina Global Screening Array (650000 SNPs); raw data were subsequently processed for quality control and normalization using R (minfi and ChAMP packages) and PLINK [3,4]. Genetic background related to hypoxia adaptation was captured using principal components from SNPs in 25 hypoxia-related genes. Factorial analysis of mixed data (FAMD) was used to summarize a wide set of variables including lifestyle, diet, psychological, anthropological, exposure-related, and immune cell components. Selected FAMD and genetic principal components were screened for collinearity and included as covariates. High-dimensional mediation analysis (HDMA) was performed to test whether DNA methylation mediates phenotypic traits related to chronic high-altitude hypoxia. The approach combined Sure Independence Screening (SIS) for CpG preselection with de- biased Lasso regression to estimate the exposure–mediator (α) and mediator–outcome (β) paths. Mediation was considered significant based on the maximum p-value from both paths,

DOI: 10.54103/2282-0930/29585

with global indirect effects calculated by summing all significant α×β products, and direct effects obtained by subtracting the total indirect effect from the total effect [5].

RESULTS

DNA Methylation significantly mediated the effect of high altitude on several traits that were hemselves significantly associated with altitude. For 24-hour heart rate, the total effect was +1.79 bpm, with 50% mediated by two CpG sites in B3GNT2 and KIAA0368. Hemoglobin increased by 3.87 g/dL, with 2% mediated by two CpG sites in SEMA4F; hematocrit increasing by 11.41 percent, with 1.7% mediated through two CpG sites in SEMA4F. Respiration rate increased by 2.22, with an inverse mediation effect of −0.21 (9%) involving three CpG sites associated with: MRPS34, EME2, and ARHGEF4. Oxygen saturation dropped by 2.2 points, 2.8% of which was mediated by an unannotated CpG. 24 hour systolic and diastolic blood pressure decreased by 8.25 and 2.21 mmHg, with 8.1% and 24% mediation through ST3GAL1 and CSGALNACT2, respectively. Finally, high-altitude exposure was associated with an estimated 80% reduction in hypertension risk (OR =0.20, 95% CI [0.078, 0.515]), with 3% of this protective effect mediated by DNA methylation at three CpG sites in ABCG1 and ARHGEF4.

CONCLUSION

These findings suggest that DNA methylation may contribute to high-altitude adaptation by modulating physiological functions, particularly those related to oxygen transport and cardiovascular regulation. In addition to quantifying mediated effects, the analysis also provided insights into the genomic context in which these effects occur, by identifying specific CpG sites within genes that may hold biological relevance. For example, ABCG1 included one of the mediating CpG sites; although this gene has been previously linked to hypertension, its role in chronic hypoxia remains to be clarified [6]. This may offer a novel entry point for future research into the molecular mechanisms of high-altitude adaptation. Other, less-characterized genes also emerged and require further investigation to understand their potential contribution.

REFERENCES

1. Bilo G., Villafuerte F.C., Zanotti L. et al., Differences in cardiovascular risk in the general population of low and high altitude residents in Peru. HIGH Altitude CArdiovascular REsearch Latin America Population Study (HIGHCARE-LAPS). European Heart Journal, 2024 Oct; 45(Suppl_1):ehae666.2944.
2. West J.B., High-altitude medicine. American Journal of Respiratory and Critical Care Medicine, 2012 Dec; 186(12):1229–1237.
3. Sahoo K., Sundararajan V., Methods in DNA methylation array dataset analysis: A review. Computational and Structural Biotechnology Journal, 2024; 23:2304–2325.
4. Purcell S., Neale B., Todd-Brown K. et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics, 2007 Sep; 81(3):559–575.
5. Clark-Boucher D., Zhou X., Du J. et al. Methods for mediation analysis with high-dimensional DNA methylation data: Possible choices and comparisons. PLoS Genet. 2023 Nov 7;19(11):e1011022.
6. Jin J, Zhao X, Zhu C et al. Hypomethylation of ABCG1 in peripheral blood as a potential marker for the detection of coronary heart disease. Clin Epigenetics. 2023 Jul 28;15(1):120. doi: 10.1186/s13148-023- 01533-6.

DOI: 10.54103/2282-0930/29585