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New genetic and physiological factors for excessive erythrocytosis and Chronic Mountain Sickness

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Villafuerte FC. New genetic and physiological factors for excessive erythrocytosis and Chronic Mountain Sickness. J Appl Physiol 119: 1481–1486, 2015. First published August 13, 2015; doi:10.1152/japplphysiol.00271.2015.—In the last few years, genetic and functional studies have provided important insight on the pathophysiology of excessive erythrocytosis (EE), the main sign of Chronic Mountain Sickness (CMS). The recent finding of the association of the CMS phenotype with a single-nucleotide polymorphism (SNP) in the Sentrin-specific Protease 1 (SENP1) gene, and its differential expression pattern in Andean highlanders with and without CMS, has triggered large interest in high-altitude studies because of the potential role of its gene product in the control of erythropoiesis. The SENP1 gene encodes for a protease that regulates the function of hypoxia-relevant transcription factors such as Hypoxia-Inducible Factor (HIF) and GATA, and thus might have an erythropoietic regulatory role in CMS through the modulation of the expression of erythropoietin (Epo) or Epo receptors. The different physiological patterns in the Epo-EpoR system found among Andeans, even among highlanders with CMS, together with their different degrees of erythropoietic response, might indicate specific underlying genetic backgrounds, which in turn might reflect different levels of adaptation to lifelong high-altitude hypoxia. This minireview discusses recent genetic findings potentially underlying EE and CMS, and their possible physiological mechanisms in Andean highlanders.

Chronic Mountain Sickness; excessive erythrocytosis; chronic hypoxia; high altitude; Andes

IN THE LAST FEW YEARS, GENETIC and functional studies have provided important insight into the pathophysiology of excessive erythrocytosis (EE) and Chronic Mountain Sickness (CMS). The use of genomic technologies has allowed the identification of functionally relevant single-nucleotide polymorphism (SNPs) as markers of “maladaptation” to life at high altitude, as well as some potential functional correlates. This minireview discusses recent genetic findings potentially underlying EE and CMS, and their possible physiological mechanisms among residents of the Andean highlands.

CHRONIC MOUNTAIN SICKNESS OR MONGE DISEASE

CMS is a clinical syndrome characterized by EE, which is defined by hemoglobin (Hb) concentration values ≥21 g/dl for men or ≥19 g/dl for women (30). CMS is diagnosed on the basis of the presence of EE accompanied by three or more of the following symptoms: breathlessness, palpitations, sleep disturbance, cyanosis, dilation of veins, paresthesia, headache, or tinnitus (29, 33, 39) according to the Qinghai International Consensus scoring system (30). Pulmonary hypertension is a frequent complication of the condition and may lead to right-heart failure in advanced stages of the disease (42, 43). CMS affects people who reside at altitudes ≥2,500 m above sea level, and it is estimated that 5-10% of the world’s population living at high altitude may develop the condition (13, 21, 30, 48, 51). In the central Andes of Peru (>4,000 m), 15–20% of the adult male population suffers from CMS, and its prevalence increases with age, rising up to 30% by the age of 50 (29, 39).

The pathophysiological mechanism of EE is still debatable; however, it is well known that hypoxemia represents its underlying stimulus because relocation of patients with CMS to sea level reverses the disorder completely. Also, it has been shown that phlebotomy, with or without isovolemic hemodilution, alleviates CMS-related symptoms, suggesting that many of these are associated with EE (37, 46, 57, 58).

A POTENTIAL GENETIC MARKER FOR EXCESSIVE ERYTHROPOIESIS

Although it has been known for some time that high Hb concentration values among Andean highlanders show heritability and familial character (4, 31), until recently no specific alleles had been found to be associated with the exaggerated red blood cell count in patients with CMS. Recent studies have
revealed that specific SNPs in the EPAS1 and EGLN1 genes are associated with low Hb concentration values in Tibetan highlanders and suggest that these markers may be linked to a physiological mechanism that confers protection to this population against the development of EE and CMS (5, 49, 62). The products of these genes, Hypoxia-Inducible Factor-2 (HIF-2) and Prolyl Hydroxylase Domain 2 (PHD2), respectively, are major regulators of erythropoiesis given their central role in the control of the hypoxic response of erythropoietin (Epo) expression at the transcriptional level. However, among Andeans, no such “protective” alleles have been identified, and no differences in the frequencies of these SNPs between healthy highlanders and those with CMS have been demonstrated (31). Differences are also absent in other candidate genes associated with erythropoiesis, such as Epo and Epo receptor (EpoR).

The recent finding of an association of the CMS phenotype among Andeans with an SNP in the sentrin-specific protease 1 (SENP1) gene has triggered large interest in the field of high-altitude studies because of the potential role of its gene product in the control of erythropoiesis (7, 47, 66). The identified candidate gene was validated by hypoxic functional testing in cultured fibroblasts derived from skin biopsies obtained from the same subjects. Compared with normoxic controls, hypoxia treatment (1.5% O2 for 24 h) induced a significant downregulation of SENP1 expression in non-CMS cells and an upregulation in CMS cells (66). Therefore, it is likely that the lower arterial O2 saturation in patients with CMS induces a higher expression of SENP1 and favors exaggerated erythropoiesis. Thus in contrast with Tibetans and Ethiopians, Andeans show a proerythropoietic SNP rather than protective SNPs against erythrocytosis. This supports the hypothesis of different degrees of high-altitude adaptation among populations. It would be interesting to explore whether the SENP1 SNP found in Tibetans and Ethiopians correspond to the “adapted variant” of the SENP1 allele found in healthy Andean highlanders.

**PHYSIOLOGICAL CORRELATES TO THE CANDIDATE GENE SENP1**

SENP1 plays important roles in the regulation of multiple cellular signaling pathways, including glucose and mitochondrial metabolism, and hormone receptor activity regulation (1, 6, 9, 11, 34, 52, 60). Also, studies in animal models show that SENP1 plays a key function in normal erythropoiesis. But is there a potential link between SENP1 and the pathophysiological erythropoietic response? SENP1 is a small ubiquitin-related modifier (SUMO) protein-specific protease that regulates the function of hypoxia-relevant transcription factors such as HIF and GATA (8, 65). SENP1 deconjugates the SUMO from HIF1α and rescues this subunit from ubiquitination and degradation (Fig. 1). Studies in mouse embryos have shown that SENP1 is required for hepatic Epo production and erythropoiesis, and that senp1−/− mice die from anemia (8). Thus although this pathway has not been yet verified in humans, it seems likely that SENP1-dependent regulation of HIF2α, the main erythropoietic HIFα isoform in adults, shares similar direct and indirect regulatory pathways with HIF1α (8, 25).

In addition, SENP1 regulates erythropoiesis via GATA-1 deSUMOylation (65). Consistently, GATA1-null mouse embryos die from severe anemia (15). The critical role of GATA1 in erythropoiesis is attributed to its activity in driving expression of many erythropoietic genes including enzymes involved in heme biosynthesis, hemoglobin, and EpoR (10, 17, 26, 67). Among these, EpoR expression has been well established to be critical for definitive erythropoiesis in murine fetal liver (28, 35, 39). Yu et al. (65) showed that a SUMOylated form of GATA1 accumulated in senp1−/− mice and correlated with a downregulation of GATA1-dependent gene expression including EpoR and β-hemoglobin. The study also showed that GATA1 can be directly deSUMOylated by SENP1, resulting in the modulation of GATA1-dependent EpoR expression and erythropoiesis.
It is interesting that these two SENP1 targets, HIF and GATA, control the Epo-EpoR system, and thus it is likely that hypoxic SENP1 upregulation as shown in patients with CMS (66) alters the Epo-EpoR system and therefore erythropoiesis.

ARE THERE ANY OTHER POTENTIAL FUNCTIONAL CORRELATES TO SENP1-INCREASED EXPRESSION IN CMS?

HIF-mediated hypoxic expression of genes such as VEGF might be enhanced by SENP1 due to deSUMOylation and increased HIFαx subunit stability (8). Apenzeller and coworkers (2) showed that VEGF expression was increased in patients with CMS, and it was positively correlated with CMS score and negatively with arterial saturation. More recently, Xu et al. (61) have provided evidence that SENP1 plays an essential role as a positive regulator of hypoxia-driven VEGF production and angiogenesis by showing that silencing of SENP1 expression decreases VEGF production and abrogates the angiogenic functions of endothelial cells. Therefore, there is a possibility that an increased SENP1 expression in CMS correlates to changes in the expression of VEGF.

THE Epo-EpoR SYSTEM AND ITS PHYSIOLOGICAL ASSOCIATION WITH EE

The role of SENP1 in erythropoiesis supposes HIF and GATA stabilization with the consequent expression of target genes. SENP-dependent (direct or indirect) stabilization of HIF2α would lead to increased Epo expression and increased erythropoiesis, similarly to what is observed in other conditions with augmented HIF2α levels (27, 44). However, elevated blood Epo values are not common in patients with CMS. Most highlanders with CMS exhibit EE but normal Epo values for the altitude of residence, similar to those observed in their healthy counterparts (16, 23, 32). Yet a subgroup of patients with CMS show significantly higher Epo values (2 SD above the altitude-normal average) usually with slightly higher Hb (12, 32, 53) (Table 1). This finding might help explain why most studies, but not all, report normal Epo average values in CMS (16, 23, 32, 45, 53), because these values would depend on the proportion of participants with normal and high Epo values. Moreover, given that increased SENP1 expression supposes increased Epo expression, it would be important to question whether the strength of the association between the SENP1 polymorphism and the CMS phenotype remains the same when the proportion of highlander participants with high Epo is changed. This is definitely an aspect that must be taken into consideration in future research. The remarkable difference in Epo levels between the two subgroups of patients with CMS suggests underlying genetic differences.

IS IT POSSIBLE THEN TO EXPLAIN AN INCREASED PRODUCTION OF RED BLOOD CELLS WITH NORMAL Epo VALUES?

Epo action depends both on Epo concentration and on the abundance and type of Epo receptors (24, 50). Thus modulation of EpoR expression or differential regulation of expression of EpoR splice variants represents a potential erythropoiesis regulatory mechanism. SENP1-mediated regulation of EpoR expression is likely to occur through GATA-1. The 5′-flanking region of the EpoR gene has several potential GATA-1 binding sites and EpoR expression is regulated by promoters that bind to GATA-1 (64, 65, 67). Also, it has been demonstrated that this regulation is specific for cells of the hematopoietic line (63).

In addition to EpoR gene expression modulation, the expressed proportion of splice variants of the gene can also affect the sensitivity of the system to Epo. EpoR can be synthesized in several forms: full-length, truncated, and soluble. Exons 1 to 5 encode the extracellular domain, exon 6 encodes the membrane-spanning domain, and exons 7 and 8 encode the intracellular domain (20). The soluble receptor form (sEpoR) with a molecular weight of 29 kDa corresponds with the extracellular domain of the complete membrane EpoR (mEpoR) (18, 41, 56), and its synthesis occurs by alternative splicing of EpoR mRNA (14, 19, 56). Recent findings suggest that the sEpoR plays a role in modulating Epo action in patients with CMS (53).

Although it is likely that genetic variation at the splicing machinery level has a significant effect on the abundance of membrane-bound or soluble Epo receptors, SUMOylation of spliceosome factors might also play an important role. Proteomic analysis has revealed that SENP isoforms are key in the deSUMOylation and function of these factors (55). Thus in addition to the role of SENP1 in EpoR expression via GATA-1, it has also a potential role modifying the splicing machinery and therefore the production of EpoR isoforms.

Functionally, several studies have shown that the binding of Epo to sEpoR limits its ability to bind mEpoR (3, 38, 50), and thus this soluble isoform acts as an Epo “buffer” regulating available circulating Epo concentration. Our group has recently shown that EE is strongly associated with low circulating sEpoR values and high Epo-to-sEpoR ratios (Epo/sEpoR), leading to elevated plasma Epo availability, and thereby a stronger stimulus for erythropoiesis (53) (Fig. 2). For this reason, when comparing healthy highlanders and those with CMS with low and high Epo levels, Hb concentration shows an exponential rise with increasing Epo/sEpoR ratio values, reaching the highest level in the high Epo group.

Table 1. Characteristics of sea-level residents, healthy highlanders, and patients with CMS

<table>
<thead>
<tr>
<th></th>
<th>Sea-Level Residents</th>
<th>Healthy Highlanders</th>
<th>Normal-Epo CMS</th>
<th>High-Epo CMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dl</td>
<td>14.5 ± 0.2</td>
<td>17.7 ± 0.2b</td>
<td>22.2 ± 0.2bc</td>
<td>24.2 ± 0.8bcd</td>
</tr>
<tr>
<td>CMS score</td>
<td>1.4 ± 0.3</td>
<td>2.7 ± 0.4a</td>
<td>7.2 ± 0.9bc</td>
<td>8.0 ± 1.4bcd</td>
</tr>
<tr>
<td>Arterial O2 sat. %</td>
<td>98.3 ± 0.2</td>
<td>88.2 ± 0.6b</td>
<td>84.4 ± 0.7bc</td>
<td>82.5 ± 1.1bc</td>
</tr>
<tr>
<td>Epo, pg/ml</td>
<td>23.6 ± 1.0</td>
<td>37.8 ± 1.9g</td>
<td>43.5 ± 2.4b</td>
<td>87.1 ± 8.4bcde</td>
</tr>
</tbody>
</table>

CMS, Chronic Mountain Sickness; Epo, erythropoietin. Sea-level residents (n = 25), healthy highlanders (n = 44), normal-Epo CMS (n = 32), and high-Epo CMS (n = 10). Values are means ± SE. Significance aP < 0.01 vs. sea-level residents, bP < 0.001 vs. sea-level residents, cP < 0.001 vs. healthy highlanders, dP < 0.001 vs. normal-Epo CMS. Data from (53).
At present, information does not exist on the effect of lifelong chronic hypoxia exposure on sEpoR. However, experiments in mice have shown that sEpoR expression can be downregulated by prolonged exposure to hypoxia (33). Changes in alternative splicing of several genes have been reported as a physiological response to hypoxia, whether by upregulating or downregulating splice isoform expression (54). The fact that the accentuated hypoxemia in patients with CMS and normal Epo compared with healthy highlanders is not associated with higher plasma Epo but with decreased sEpoR concentration suggests that chronic hypoxia affects Epo and sEpoR in CMS in differential manners. Whether reduced blood sEpoR levels result from a chronic downregulation of sEpoR production at the mRNA splicing machinery level caused by the chronically lower arterial O₂ saturation in patients with CMS or from genetic differences has yet to be clarified. Also, it is unknown whether differential expression of the splice EpoR isoforms, sEpoR mEpoR, contributes to increased Epo action in persons with similar Epo values, and also whether factors such as SENP1 are involved in EpoR expression and the balance between mEpoR and sEpoR. It is possible that reduced sEpoR levels in CMS are accompanied by increased levels of its membrane counterpart leading to increased sensitivity to Epo in addition to increased Epo availability. Finally, EpoR expression and the relative abundance of its splice isoforms also might be influenced by epigenetic factors. Several studies have suggested that perinatal hypoxia, for example, predisposes highlanders to develop EE and CMS during adulthood (22, 23, 36, 40). Whether perinatal hypoxia or other environmental factors affect the Epo-EpoR system is one important aspect that must be considered in future research.

The different physiological patterns in the Epo-EpoR system among Andean highlanders together with their different degrees of erythropoietic response might indicate specific underlying genetic backgrounds, which in turn, might reflect different levels of adaptation to chronic high-altitude hypoxia. Patients with CMS and high Epo, with their exaggerated erythrocytosis and elevated plasma Epo, could be regarded as the least adapted, followed by highlanders with CMS and normal Epo, with still excessive but lower Hb and normal circulating Epo concentration, and finally healthy Andeans, with a modestly elevated Hb and normal Epo values for the altitude of residence, as the most adapted among groups within the same population.

Therefore, functional and phenotypic differences between healthy Andean highlanders and those with CMS, and even among patients with CMS, suggest the involvement of multilevel physiological control points, each with a potential specific combination of genetic variants. These variants would ultimately determine the presence and severity of EE and the degree of maladaptation to chronic high-altitude hypoxia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

F.C.V. conception and design of research; F.C.V. performed experiments; F.C.V. analyzed data; F.C.V. interpreted results of experiments; F.C.V. drafted manuscript; F.C.V. edited and revised manuscript; F.C.V. approved final version of manuscript.

REFERENCES

Enzyme-linked immunosorbent assay (ELISA) is a commonly used technique for detecting and quantifying specific antigens or antibodies in biological samples. This method relies on the specific binding of an antigen to a solid substrate, followed by the detection of bound antibodies using a labeled secondary antibody.

Erythropoietin (Epo) plays a crucial role in the regulation of red blood cell production. It is produced in response to hypoxia and acts on the erythroid progenitor cells to stimulate erythropoiesis.

Hypoxia-induced SUMOylation of E3 ligase HAF determines specific interactions with EpoR and contributes to abnormities of mitochondria and cardiomyopathy. These findings highlight the importance of understanding the molecular mechanisms underlying hypoxia and its physiological consequences.

High serum Epo levels are associated with excessive erythrocytosis, a condition characterized by an abnormally high number of red blood cells. This condition can be caused by various factors, including altitude exposure, chronic diseases, and genetic predispositions.

The role of estrogen receptor-alpha in the regulation of erythropoiesis is also discussed. Estrogen receptor-alpha is involved in the control of red blood cell production and can be upregulated by estrogen receptor gene disruption.

In summary, the review presents a comprehensive overview of the molecular biology of erythropoiesis, highlighting the complex interplay between various factors that influence red blood cell production and function.


