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Brief Reports

Genetic Variation in *SENP1* and *ANP32D* as Predictors of Chronic Mountain Sickness

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Abstract

Cole, Amy M., Nayia Petousi, Gianpiero L. Cavalleri, and Peter A. Robbins Genetic variation in *SENP1* and *ANP32D* as predictors of chronic mountain sickness. *High Alt Med Biol* 15:497–499, 2014.—Chronic mountain sickness (CMS) is a serious illness that affects life-long high-altitude residents. A recent study analyzed whole genome sequence data from residents of Cerro de Pasco (Peru) in an effort to identify the genetic basis of CMS and reported *SENP1* (rs7963934) and *ANP32D* (rs72644851) to show signatures consistent with natural selection and protective against CMS (Zhou et al. 2013). We set out to replicate these observations in two Andean cohorts from Cerro de Pasco, consisting of 84 CMS cases and 91 healthy controls in total. We report evidence of association for rs7963934 (*SENP1*) in the combined cohorts (meta-analysis $p = 8.8 \times 10^{-4}$ OR 2.91, CI 1.56–5.5, I = 0). The direction of effect was the same as in the original publication. We did not observe any significant correlation between rs72644851 (*ANP32D*) and the CMS phenotype, within or across cohorts (meta-analysis $p = 0.204$, OR 1.37, CI 0.84–2.241, I = 0). Our results provide independent evidence in support of a role for *SENP1* in CMS in individuals of Quechua ancestry and suggest the *SENP1* and *ANP32D* signatures of selection are in tight linkage disequilibrium (LD).

Introduction

Chronic mountain sickness (CMS, also known as Monge's disease) is a serious condition that occurs in life-long high altitude residents who are mal-adapted to their hypobaric environment (Monge, 1942; Monge et al., 1992; Xing et al., 2008). CMS is predominantly characterized by excessive erythrocytosis (hemoglobin concentration ≥21 g/dL for males and ≥19 g/dL for females) and hypoxemia. The condition can lead to pulmonary hypertension and cor pulmonalae (Leon-Velarde et al., 2005).

The incidence of CMS varies across indigenous high-altitude populations, with a higher prevalence observed in Quechua Andeans (around 15%) in comparison to Han Chinese (5.6%) and Tibetans (1.2%) resident at similar altitudes (Leon-Velarde et al., 2000; Mejia et al., 2005; Wu, 2005). A potential explanation for these observations would be differences in the frequency of relevant underlying genetic factors across Andean, Tibetan, and Han populations.

Recent work by Zhou et al. (2013) aimed to identify the genetic basis of CMS in an effort to understand what makes some individuals more or less susceptible to developing this life-threatening condition. They compared whole genome sequence data of 10 Peruvian individuals (recruited from Cerro de Pasco) with CMS to 10 without CMS, and reported 11 regions across the genome that contained haplotypes that were both enriched in non-CMS individuals and illustrated signatures characteristic of recent natural selection. Two of these regions appeared significant (in the context of formal tests of selection) in a comparison of non-CMS versus either CMS or other matched population controls. One region contained the genes *SENP1,* an erythropoiesis regulator (Yu et al., 2010), and the other contained *ANP32D,* an oncogene (Kadkol et al., 1999). The authors validated the frequencies of these two haplotypes by conducting genotyping of two variants (rs7963934 (*SENP1*) and rs72644851 (*ANP32D*)) in an additional 10 case and 10 control subjects. These two variants were reported to be in the ''primary differential haplotype blocks,'' that is, the variants defined the two haplotypes that appeared enriched in the non-CMS population and protective against the condition.

Independent replication is an important component of genetic mapping. In this context, we set out to replicate the correlation reported by the authors between these two variants and CMS status.

Materials and Methods

The Oxford Tropical Research Ethics Committee and the Universidad Peruana Cayetano Heredia Research Ethics

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Table 1. Genotypic and Allelic Contingency Tables for rs7963934 (*SENP1*)

Cohort A	*Cases*	*Controls*	*Cohort B*	*Cases*	*Controls*
GG	29 (0.58)	42 (0.84)	GG	21 (0.62)	35 (0.85)
GC	19 (0.38)	7 (0.14)	GC	12 (0.35)	5 (0.12)
CC	2 (0.04)	1 (0.02)	CC	1 (0.03)	1 (0.02)
Genotypic P *value*	*0.008*		*Genotypic* P *value*	*0.039*	
G	77 (0.77)	91 (0.91)	G	54 (0.79)	75 (0.91)
C	23 (0.23)	9 (0.09)	C	14 (0.21)	7 (0.09)
Allelic P *value*	0.0113		*Allelic* P *value*	0.0567	

Figures in brackets represent frequencies.

Committee approved the studies, which were conducted in accordance with the Declaration of Helsinki.

Cohort description

We considered two CMS case-control cohorts of indigenous Andean ancestry, recruited from Cerro de Pasco, Peru. Cohort A consisted of 50 cases and 50 controls and was collected in 2008. Cohort B consisted of 34 cases and 41 controls and was collected in 2012. All individuals were male and to the best of our knowledge, were unrelated to at least one degree. Participants were included following accepted diagnostic criteria (as per Leon-Velarde et al., 2005).

The Qinghai CMS score questionnaire was completed on all individuals in order to assess CMS diagnosis. Scores from 0–3 were assigned to clinical symptoms such as breathlessness and/or palpitations, sleep disturbance, cyanosis, dilation of veins, paresthesia, headache, and tinnitus. Those with a CMS score >11 were defined as cases, and <5 as controls (Leon-Velarde et al., 2005).

For inclusion as a CMS case, patients must have: i) been born at high altitude; ii) have previously been diagnosed with CMS; and iii) have a measured hematocrit of 63% or above on the day of data collection to compliment the diagnostic criterion of hemoglobin (Hb) >21 g/dL for males. For inclusion as a control, patients must have: i) been born at high altitude; ii) have no previous diagnosis of CMS; and iii) have a measured hematocrit of 54% or below on the day of DNA collection.

The average age in years of cases and controls in Cohort B was 44.6 ($\pm$12.4) and 43.7 ($\pm$9.5) years, respectively. Body mass index values were 26.9 kg/m2 ($\pm$3.6) for cases and 25.0 kg/m2 ($\pm$2.4) for controls. Comparable data for Cohort A were not recorded at the time of recruitment.

Genotyping and analysis

TaqMan SNP genotyping assays (Life Technologies, Carlsbad, CA) were applied for the two SNPs, rs7963934 (*SENP1*) and rs72644851 (*ANP32D*) following standard protocols. Alleles reported in contingency tables are from the sense (+ve) strand of the February 2009 Human Genome Reference (Hg19). Significance within each cohort was determined using the Fishers exact test values applied to genotypic and allelic contingency tables. Significance across combined cohorts was determined through fixed effects meta-analysis (performed using PLINK v1.07) (Purcell et al., 2007).

Results

Genotype counts are illustrated in Table 1 for rs7963934 and Table 2 for rs72644851. Both variants satisfied the Hardy-Weinberg equilibrium.

There was some evidence of association for rs7963934 (*SENP1*) with CMS in both cohorts when considered independently. The direction of effect was the same as in the original publication. Meta-analysis across our two cohorts resulted in a p value of 8.8×10^{-4} (OR 2.91, CI 1.56- 5.5, $I=0$).

We did not observe any significant correlation between rs72644851 (*ANP32D*) and the CMS phenotype, within or across cohorts (meta-analysis $p=0.204$, OR 1.37, CI 0.84–2.241, $I=0$).

Given the relatively close proximity of these variants on chromosome 12 (they are 332 kb apart), we calculated (using our combined case and control genotypes) linkage disequilibrium values betweem them. Results showed they were highly correlated, with an r^2 value of 0.575 and a D' of 1. Calculations in Mexican descent subjects of the 1000 Genomes Project are comparable ($r^2=0.522$, D' $=0.910$). These

Table 2. Genotypic and Allelic Contingency Tables for rs72644851 (*ANP32D*)

Cohort A	*Cases*	*Controls*	*Cohort B*	*Cases*	*Controls*
GG	24 (0.49)	33 (0.67)	GG	16 (0.49)	24 (0.62)
GA	23 (0.47)	12 (0.24)	GA	15 (0.45)	11 (0.28)
AA	2 (0.04)	4 (0.08)	AA	2 (0.06)	4 (0.10)
Genotypic P *value*	0.064		*Genotypic* P *value*	0.323	
G	71 (0.73)	78 (0.8)	G	47 (0.71)	59 (0.76)
A	27 (0.28)	20 (0.2)	A	19 (0.29)	19 (0.24)
Allelic P *value*	0.3155		*Allelic* P *value*	0.5739	

Figures in brackets represent frequencies.

results suggest that the two haplotypes reported by Zhou et al. (2013) (containing *SENP1* and *ANP32D*) are very strongly linked and should be interpreted accordingly.

Discussion

Our results provide independent evidence in support of a role for *SENP1* in CMS, as proposed by Zhou et al. (2013). Although we did not observe a sigificant association result for the *ANP32D* variant, the high levels of LD between this and the *SENP1* variant suggest that, assuming the effect on CMS is real, increasing cohort size would eventually generate a significant result. Indeed, the fact that these two variants are in high LD suggests that the two haplotypes reported by Zhou et al. are very closely related and perhaps should be considered as one broad signal of selection.

Several factors must be considered when interpreting our results. Although the subjects in both of our cohorts were recruited from Cerro de Pasco (the same town as those in Zhou et al.), in the absence of GWAS data, we cannot rule out overlap between our subjects and those in Zhou et al. However, the case and control numbers in our study were several times ($\sim$8X) greater, which provides a considerable degree of independence to our dataset. In this context our study was well positioned to replicate any real effects for these specific variants.

Second, as we were working with existing cohorts and associated phenotypic information, we were unable to match exactly the phenotype used by Zhou et al. However for the purpose of this report, they were well-matched for the criterion of excessive erythrocytosis, the key diagnostic feature of CMS.

SENP1 has been shown to regulate erythropoietin (EPO) production by regulating the stability of hypoxia-inducible factor 1α (HIF1α) during hypoxia (Yu et al., 2010) and indeed *SENP1*$^{-/-}$ mice die of anemia in early life (Cheng et al., 2007; Yu et al., 2010). *SENP1* also mediates a positive feedback loop in hypoxic conditions that is responsible for vascular endothelial growth factor (VEGF) production and angiogenesis (Xu et al., 2010). Given that excessive erythrocytosis is the predominant feature of CMS, it is of interest that genetic variation in *SENP1* may be implicated in its pathogenesis. In contrast, little is known about the functional relevance of *ANP32D,* and given the extensive LD across the region, it is probable that the signal is being driven by *SENP1*.

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