PNAS LETTER

An intronic polymorphism associated with 2,3-bisphosphoglycerate levels in human red cells is linked to expression of RhCE blood groups

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We read with interest how D'Alessandro et al. recently investigated the genetic underpinnings of the metabolic adaptations in red blood cells (RBCs) from healthy humans under high-altitude hypoxia (1). The authors concur previous in vivo studies in that 2,3-biphosphoglycerate (BPG) levels increase under high-altitude hypoxic conditions through their RBC metabolomics analysis of six healthy volunteers, who climbed to the highest city worldwide, La Rinconada, Peru (5,100 m above sea level). Strikingly, their RBC proteomics revealed the RhCE protein levels were most affected by ascent, acclimatization, and descent. Following this, metabolite quantitative trait loci analysis of 2,3-BPG using the omics and genotyping data from the Recipient Epidemiology and Donor Evaluation Study (REDS) was performed. A significant association between 2,3-BPG and genetic polymorphisms from chromosome 1 was found, and the polymorphism ranking highest in association was rs636889 located in intron 5 of *RHCE*.

The obvious question to ask is how a noncoding variant, rs636889, and/or other *RHCE* polymorphisms impact the RhCE protein to become a critical determinant of 2,3-BPG levels in RBCs of healthy volunteers? As D'Alessandro et al. mentioned, the *RH* genes comprise substantial genetic heterogeneity. We noted additional polymorphisms in linkage disequilibrium (LD) with rs636889 are present in the highly homologous *RHD* gene, which arose from a duplication event of *RHCE* (2). The *RHD* gene has single nucleotide variants (SNVs) distributed throughout the gene, including introns, depending on the phenotypic combination of Rh blood group antigens RhD, RhC, RhE, Rhc, and Rhe (3).

Similarly, in *RHCE*, more than one SNV can be used to predict the phenotype for C and c antigens, while one SNV in exon 5 defines the E and e antigens. LD analysis using LDlink (4) showed rs636889:C to correlate with SNVs encoding the RhC (rs586178:G, rs61777615:A) and Rhe (rs609320:C) antigens, whereas rs636889:T correlated with the Rhc (rs586178:C, rs61777615:G) and RhE antigens (rs609320:G; Fig. 1) (5, 6) in most populations. Lower linkage equilibria in African populations may be due to their unique diversity of *RHCE* alleles that affects the LD algorithm. Otherwise, in general, LDlink showed rs636889 travels with C/c and E/e antigen-encoding SNVs.

Although all volunteers were reported as Rh-positive, their RhCE phenotypes were not specified (1). Interestingly, Rh antigen densities are known to vary depending on the combination of C, E, c, and e antigens (8). Computational modeling of these antigen combinations showed varying degrees of RhCE extracellular loop exposure, conformational structures, and interactions with the Rh-associated glycoprotein (RhAG) (9). With RhCE playing a structural role in the band 3-macrocomplex, a recognized participant of RBC oxygen regulation (10), it would be tempting to explore whether the RhD and RhCE phenotype plays a role in or can predict 2,3-BPG levels in the REDS study. Different RhCE protein isoforms could impact the structural stability or density of neighboring molecules in this macrocomplex implicated in ammonia or carbon dioxide transport.

In conclusion, we propose that an improved understanding of 2,3-BPG heterogeneity between humans may be gained by investigating the role of Rh phenotypes and their interactions within/around the macrocomplex.

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Competing interest statement: J.R.S. is the Senior Vice President for International Society of Blood Transfusion (ISBT) and on the ISBT Board of Directors. J.R.S. and M.L.O. has given educational lectures in exchange for honoraria from Biorad and QuidelOrtho. J.R.S. and M.L.O. are inventors on patents about blood group genotyping. J.R.S. and M.L.O. own 50% each of the shares in BLUsang AB, an incorporated consulting firm, which receives royalties for said patents.

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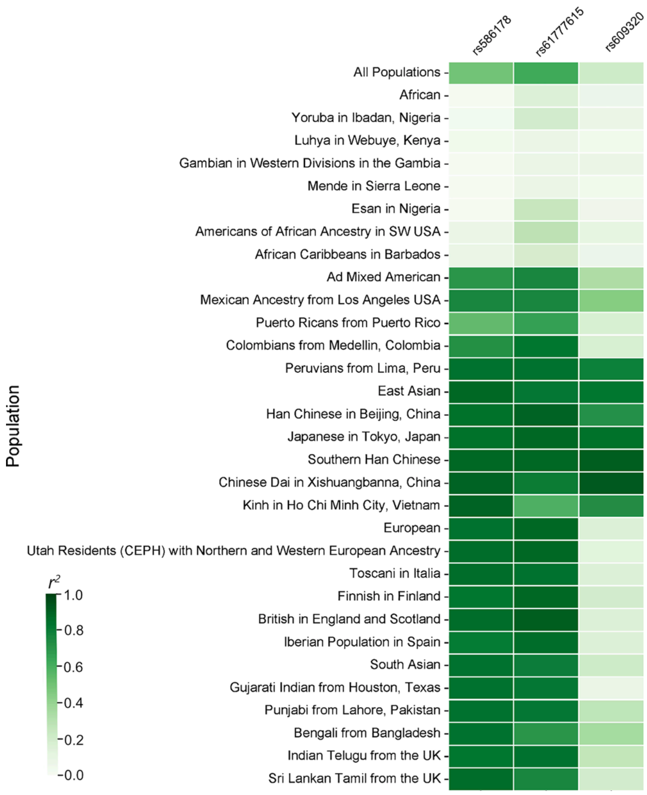

ALLELE FREQUENCY

		Top SNV from D'Alessandro *et. al.* rs636889		c.48 C/c SNV in *RHCE* exon 1 rs586178		c.336-2850 C/c SNV in *RHCE* intron 2 rs61777615		c.676 E/e SNV in *RHCE* exon 5 rs609320	
Abbrev	**n=**	**C**	**T**	**G**	**C**	**G**	**A**	**C**	**G**
ALL	2504	0.56	0.44	0.60	0.40	0.54	0.46	0.86	0.14
AFR	661	0.40	0.60	0.52	0.48	0.91	0.09	0.92	0.08
YRI	108	0.43	0.57	0.50	0.50	0.87	0.13	0.92	0.08
LWK	99	0.44	0.56	0.49	0.51	0.95	0.05	0.95	0.05
GWD	113	0.41	0.59	0.62	0.39	0.93	0.07	0.92	0.08
MSL	85	0.41	0.59	0.52	0.48	0.95	0.05	0.95	0.05
ESN	99	0.34	0.66	0.48	0.52	0.89	0.11	0.90	0.10
ASW	61	0.43	0.57	0.50	0.50	0.83	0.17	0.89	0.11
ACB	96	0.37	0.63	0.53	0.47	0.90	0.10	0.91	0.09
AMR	347	0.52	0.48	0.55	0.45	0.51	0.49	0.77	0.23
MXL	64	0.56	0.44	0.59	0.41	0.42	0.58	0.74	0.26
PUR	104	0.49	0.51	0.52	0.48	0.58	0.42	0.85	0.15
CLM	94	0.53	0.47	0.56	0.44	0.52	0.48	0.86	0.14
PEL	85	0.52	0.48	0.55	0.45	0.47	0.53	0.58	0.42
EAS	504	0.76	0.24	0.78	0.22	0.26	0.74	0.80	0.20
CHB	103	0.68	0.32	0.71	0.29	0.33	0.67	0.75	0.25
JPT	104	0.69	0.31	0.73	0.27	0.33	0.67	0.73	0.27
CHS	105	0.81	0.19	0.82	0.18	0.20	0.80	0.83	0.17
CDX	93	0.81	0.19	0.82	0.18	0.19	0.81	0.82	0.18
KHV	99	0.83	0.17	0.82	0.18	0.25	0.75	0.87	0.13
EUR	503	0.45	0.55	0.47	0.53	0.57	0.43	0.84	0.16
CEU	99	0.44	0.56	0.47	0.53	0.58	0.42	0.86	0.14
TSI	107	0.46	0.54	0.48	0.52	0.55	0.45	0.85	0.15
FIN	99	0.43	0.57	0.44	0.56	0.59	0.41	0.80	0.20
GBR	91	0.46	0.54	0.49	0.51	0.54	0.46	0.85	0.15
IBS	107	0.44	0.56	0.48	0.52	0.58	0.42	0.84	0.16
SAS	489	0.69	0.31	0.69	0.31	0.35	0.65	0.91	0.09
GIH	103	0.64	0.36	0.63	0.37	0.40	0.60	0.95	0.05
PJL	96	0.65	0.35	0.64	0.36	0.39	0.61	0.87	0.13
BEB	86	0.78	0.22	0.79	0.21	0.24	0.76	0.91	0.09
ITU	102	0.68	0.32	0.71	0.29	0.35	0.65	0.89	0.11
STU	102	0.70	0.30	0.70	0.30	0.34	0.66	0.92	0.08

Fig. 1. Linkage disequilibrium patterns and allele frequencies from LDpop analysis for rs636889 and three SNVs implicated in expression of RhCE antigens. For each population, r^2 values were plotted in a matrix heatmap using SRplot (7). The nucleotide changes linked with rs636889:C are underlined and those linked with rs636889:T are not. The nucleotide present in the reference allele for a given rs number is on the *Left* and the alternate allele on the *Right*.

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