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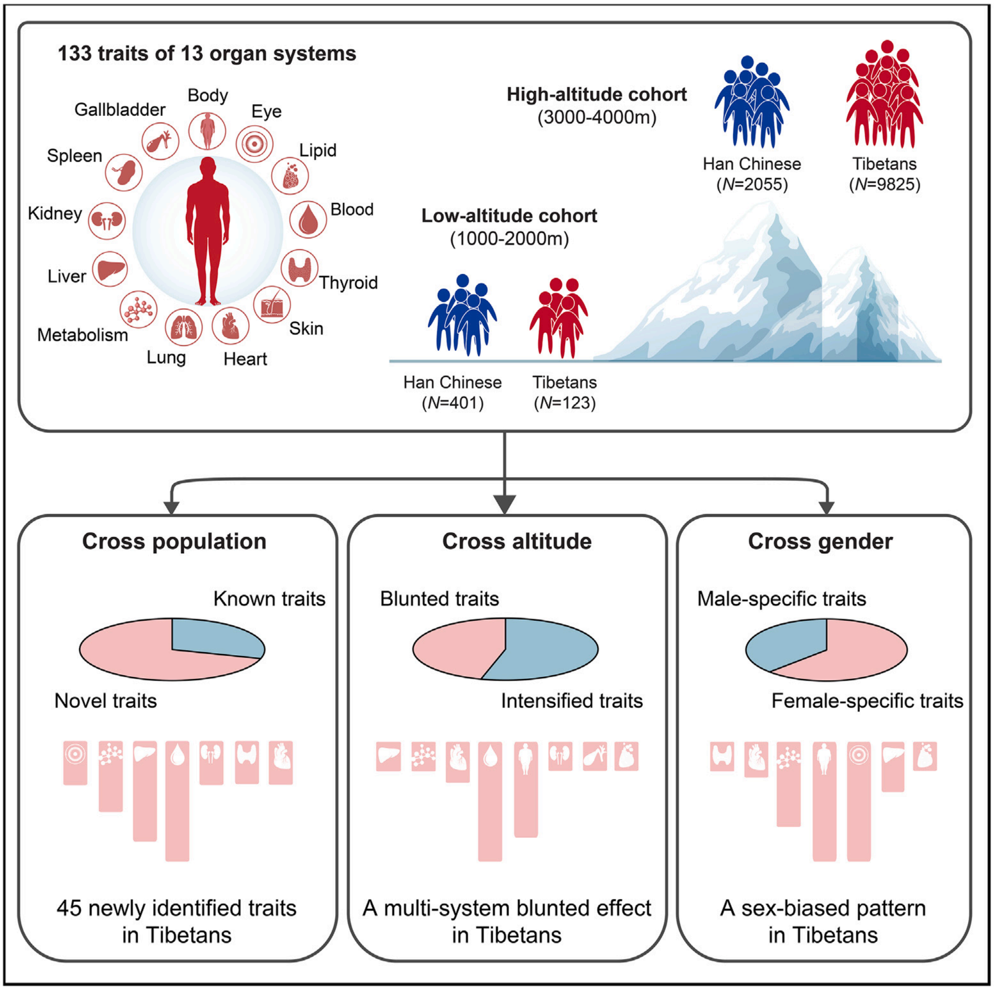

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Highlights

He et al. performed systematic phenotyping of 133 traits on 11,880 highlanders

They provided a phenotypic atlas of altitude adaptation and acclimatization

They established a landscape of adaptive phenotypes of native Tibetans

They observed a sex-biased pattern between altitude acclimatization and adaptation

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Deep phenotyping of 11,880 highlanders reveals novel adaptive traits in native Tibetans

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SUMMARY

Tibetans are the ideal population to study genetic adaptation in extreme environments. Here, we performed systematic phenotyping of 11,880 highlanders, covering 133 quantitative traits of 13 organ systems. We provided a comprehensive phenotypic atlas by comparing altitude adaptation and altitude acclimatization. We found the differences between adaptation and acclimatization are quantitative rather than qualitative, with a whole-system "blunted effect" seen in the adapted Tibetans. We characterized twelve different functional changes between adaptation and acclimatization. More importantly, we established a landscape of adaptive phenotypes of indigenous Tibetans, including 45 newly identified Tibetan adaptation-nominated traits, involving specific changes of Tibetans in internal organ state, metabolism, eye morphology, and skin pigmentation. In addition, we observed a sex-biased pattern between altitude acclimatization and adaptation. The generated atlas of phenotypic landscape provides new insights into understanding of human adaptation to high-altitude environments, and it serves as a valuable blueprint for future medical and physiological studies.

INTRODUCTION

The successful colonization of high-altitude regions represents one of the most impressive accomplishments of modern humans in recent evolutionary history given the extreme environmental conditions at highland, mainly hypobaric hypoxia and strong ultraviolet radiation that are hard to overcome with traditional means. To this end, the indigenous highland populations serve as an ideal model of understanding how natural selection shapes phenotypic adaptation in humans.[1,2] Tibetans are one of the well-known indigenous highland populations, and they are the earliest colonizers (>30,000 years ago) at the Qinghai-Tibetan Plateau (average elevation >4,000 m).[3–6] Tibetans have acquired a set of adaptive physiological characteristics to cope with high-altitude challenges.[2,7–9] Such phenotypic adjustments of the Tibetan natives are defined as "altitude adaptation", i.e., the long-term permanent adjustments of phenotypes favored by natural selection.[10] Comparatively, the phenotypic adjustments of highland migrants are called "altitude acclimatization", i.e., the temporary and reversible adjustments of physiological phenotypes after moving to high altitude.[11]

Many efforts have been made in characterizing the physiological similarities and differences between altitude adaptation and acclimatization. However, much remains to be elucidated. In particular, how many body systems are involved and which part plays the vital role in the adaptation and/or acclimatization are yet to be explored. In general, the previous studies have the following limitations: (1) small sample size and limited geographic coverage of highland populations, leading to a poor repeatability of the findings[12]; (2) only a small portion of phenotypes were studied, mainly restricted on the cardiopulmonary system and the blood system[13]; (3) lack of comparable samples of highland migrants who live at the same altitude with the highland natives.[14] These limitations call for systematic phenotypic profiling and large-scale comparative analyses of highland populations.

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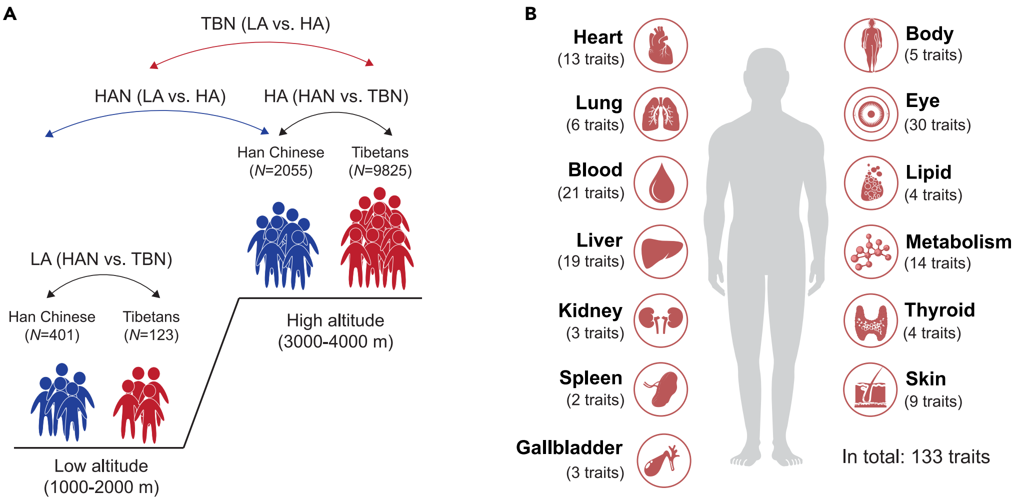


Figure 1. Schematic overview of the study design

(A) The sampling strategy of the low-altitude and the high-altitude cohorts. Tibetans (TBN) and Han Chinese (HAN) are labeled in red and blue, respectively. The compared population pairs and the sample sizes (*N*) are indicated. LA, low altitude; HA, high altitude.

(B) The 133 investigated quantitative traits that are classified into 13 organ systems.

To characterize the entire spectrum of altitude adaptation and acclimatization, and to discover previously unknown adaptive phenotypes of the highland natives, we conducted systematic phenotyping of 11,880 highlanders by inspecting 133 quantitative traits of 13 organ systems. We constructed an atlas of adaptation-acclimatization comparison and identified many novel adaptive phenotypes in Tibetans.

RESULTS

Large-scale sampling and phenotyping of native and acclimatized highlanders

We collected samples of the high-altitude (HA) cohort from 10 different geographic sites at the Qinghai-Tibetan Plateau in China, including 11,880 participants (9,825 Tibetans and 2,055 Han Chinese) with altitudes ranging from 3,560 to 3,970 m (Figure S1; Table S1). The Tibetans are native highlanders, and the Han Chinese migrants had lived at the sampled elevations for more than one year. Consequently, they represent two different groups of people subject to high-altitude stress, the altitude-adaptation population (native Tibetans), and the altitude-acclimatization population (Han migrants) (Figure 1A). In addition, as reference, we also collected samples of a low-altitude (LA) cohort, including 524 participants (123 Tibetans and 401 Han Chinese) who live at three geographic sites with altitudes ranging from 1,120 to 1,890 m (Figure S1; Table S1). Using a series of stringent quality controls for the HA and LA cohorts, 10,829 subjects with clean data were retained for downstream analyses, including 9,274 Tibetans and 1,555 Han Chinese (see STAR methods).

For phenotyping, a total of 133 quantitative traits were measured. These traits involve 13 organ systems: heart (13 traits), lung (6 traits), blood (21 traits), metabolism (14 traits), eye (30 traits), skin (9 traits), liver (19 traits), kidney (3 traits), body (5 traits), spleen (2 traits), gallbladder (3 traits), thyroid (4 traits), and lipid (4 traits) (Figure 1B; Table S2). Notably, 60 traits (45%) are firstly reported in the Tibetan populations (Table S2).

Comparison of the phenotypic patterns between altitude adaptation and acclimatization

To reveal the similarities and differences between altitude adaptation and acclimatization, we conducted analyses of four pairwise comparisons: cross-population comparisons at LA and HA, and cross-altitude comparisons of Tibetans and Han Chinese, respectively (Figure 1A). A total of 9155 HA Tibetans, 1167 HA Han Chinese, 119 LA Tibetans, and 388 LA Hans were included in the analyses (Table S1). We included 133 traits (with 87 independent traits) in the HA cohort, and 74 available traits (a subset of the 133 traits) in the LA cohort (Table S2).

We adopted a covariate-adjusted regression linear model to evaluate the significance of difference (STAR methods), and a trait was defined as a significantly differential trait (SDT) when the adjusted p-value was smaller than 0.05. To exclude the bias from sample imbalance in each sampling location, we also included the altitude as a covariate in the model (STAR methods). In the cross-population comparisons, we identified 20 SDTs out of the 74 traits between Tibetans and Han at low altitude, and 64 SDTs out of the 133 traits (40 SDTs out of the 74 traits) between Tibetans and Han at high altitude (Figure 2A; Table S2). Notably, we found 52 traits being HA-specific SDTs between populations, involving almost all the investigated organ systems, especially blood, metabolism, liver, heart, and eye (Figure 2). The apparently more cross-population SDTs in the HA cohort compared to those in the LA cohort (48% vs. 27%) suggest a remarkable effect of high-altitude environment to body system. By contrast, there were only 8 traits being LA-specific SDTs between populations, mainly related with liver function. There are 12 shared SDTs between the LA cohort and the HA cohort, mostly among the blood traits (Figures 2B and 2C).

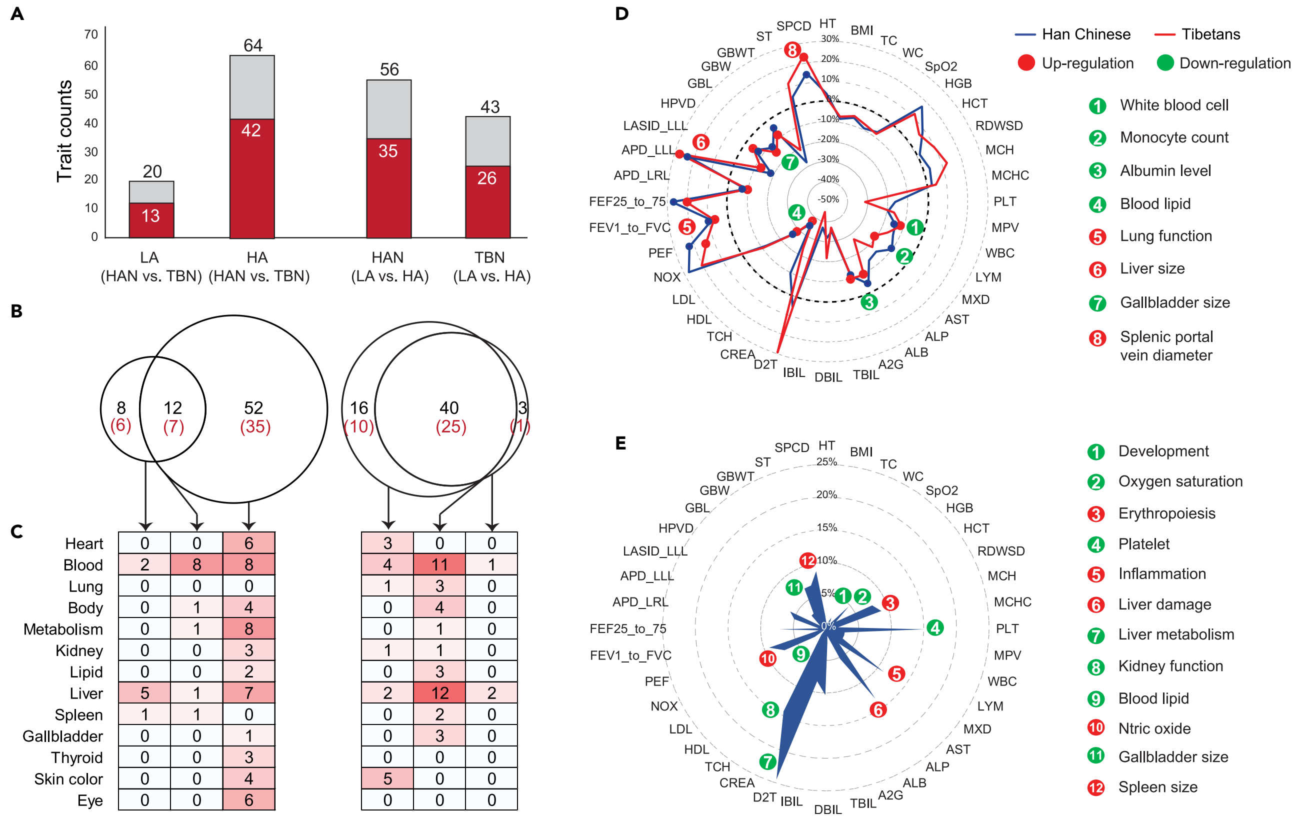


Figure 2. Phenotypic comparison between altitude adaptation and acclimatization

(A) Counts of the significantly differential traits (SDTs) among the four pairwise comparisons: the cross-population comparisons at LA (low altitude: 1000–2000 m) and HA (high altitude: 3000–4000 m), and the cross-altitude comparisons of Tibetans (TBN) and Han Chinese (HAN), respectively. The counts of independent SDTs were shown using boxes in red.

(B) Venn plots show the intersections of the two phenotypic sets from the cross-population comparisons (left panel) and the cross-altitude comparisons (right panel). The independent SDTs were also added in the Venn plots using bracketed numbers in red.

(C) The heatmaps show the phenotype counts in Panel-B when classified into 13 organ categories.

(D) The radar plot shows the difference percentages of the 40 SDTs when comparing LA and HA in Tibetans (red line) and Han Chinese (blue line), respectively. The upregulation and downregulation indexes are numerically labeled with circles in red and green, respectively. The corresponding functional interpretations are listed on the right side.

(E) The radar plot shows the difference percentages of the 40 SDTs between the Tibetan changes and the Han changes (STAR methods). The 12 functional categories with significant differences between altitude acclimatization and adaptation are labeled with circles and listed on the right side.

In the cross-altitude comparisons, we identified 56 and 43 SDTs out of the 74 traits in Han and Tibetans, respectively (Figure 2A; Table S2). The fewer SDTs in Tibetans imply a physiological "blunted effect" of the body system in the adapted population compared to the acclimatized population. Consistently, there were more acclimatization-specific SDTs in Han (16 SDTs) than the adaptation-specific SDTs in Tibetans (3 SDTs) (Figure 2B). Interestingly, there were 40 (71%) shared cross-altitude SDTs between Han and Tibetans, indicating that the majority of the physiological adjustments were similar between altitude adaptation and acclimatization. These shared SDTs mainly involve blood and liver functions (Figure 2C). The pattern was similar when only the independent SDTs were considered (Figure 2B).

Furthermore, for the 40 shared cross-altitude SDTs between Han and Tibetans, we identified 8 similar changes between adaptation and acclimatization, with the same change direction and similar magnitudes ($p > 0.05$ in Tibetan-Han comparison of HA cohort) between Tibetans and Han from LA to HA, including 5 downregulation indexes (white blood cell, monocyte count, albumin level, blood lipid, and gallbladder size) and 3 upregulation indexes (lung function, liver size, and splenic portal vein diameter) (Figure 2D). Consequently, these body systems and the relevant indexes were more responsive to high-altitude stress in both adaptation and acclimatization.

Remarkably, there were 12 significant differences ($p < 0.05$ in Tibetan-Han comparison of HA cohort) between adaptation and acclimatization, including the functional processes of development, oxygen saturation, erythropoiesis, platelet, inflammation, liver damage, liver metabolism, kidney function, blood lipid, nitric oxide, gallbladder, and spleen size (Figures 2E and S2). Importantly, the differences in kidney function, blood lipid, gallbladder wall thickness, and spleen thickness between adaptation and acclimatization are firstly identified in this study. Taken together, we demonstrate that the differences between adaptation and acclimatization are quantitative rather than qualitative. Native Tibetans and Han migrants share the majority of the phenotypic changes with the same direction, but with a different magnitude.

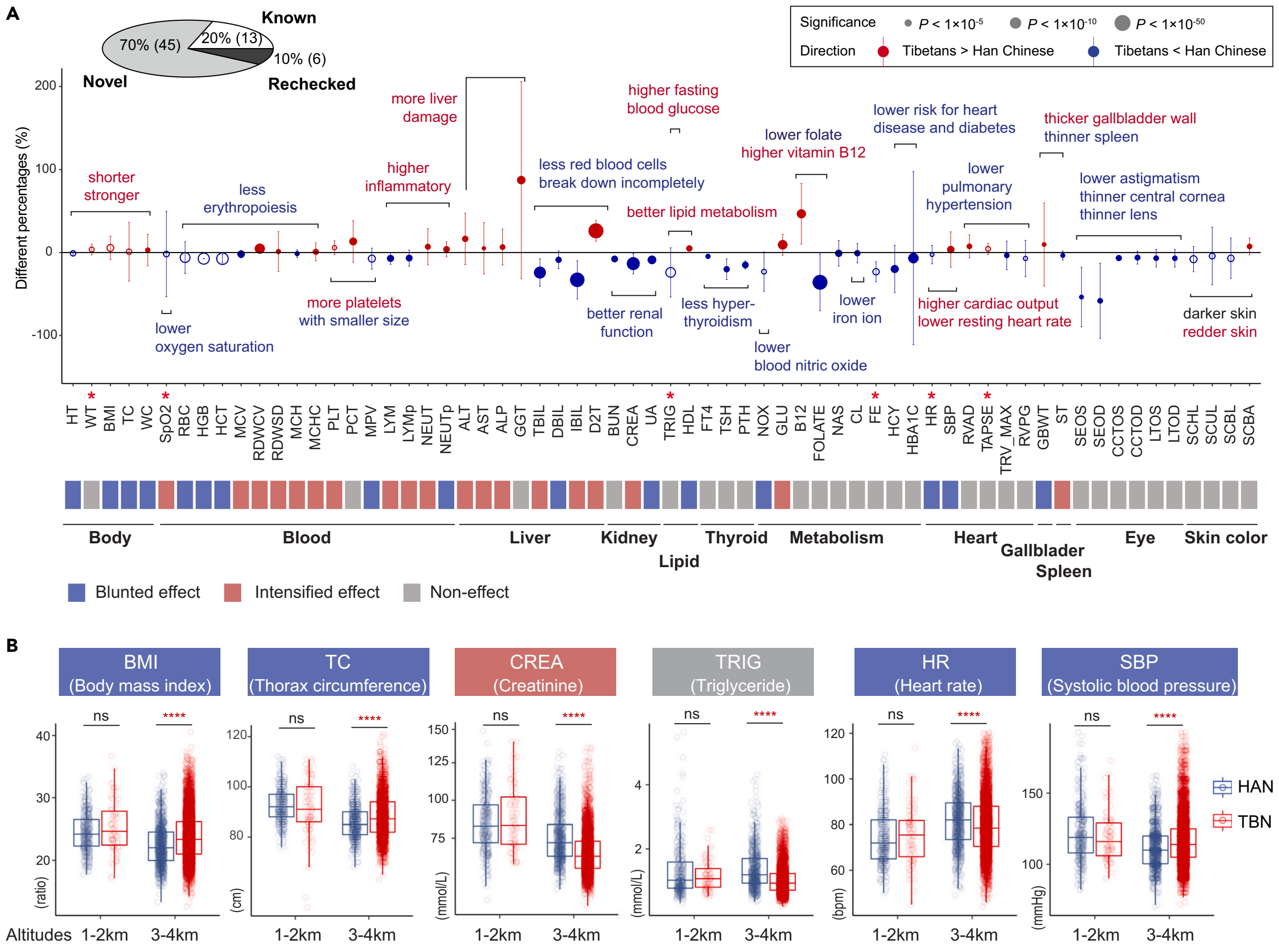


Figure 3. The newly identified adaptive traits in native Tibetans at high altitude

(A) The patterns of the 64 Tibetan adaptation-nominated traits (TANTs). The pie plot shows the counts and proportions of novel/known/rechecked traits among the 64 TANT. The upper panel indicates the different percentage of each trait between Tibetans and Han at high altitude (3000–4000 m). The solid and hollow circles represent the newly identified TANTs and the known TANTs, respectively; the rechecked TANTs are marked by red asterisks. The Tibetan-higher (in deep-red) and -lower (in blue) TANTs are highlighted. The simplified interpretations of TANTs are marked in the dot plot. The bottom panel shows the adaptive patterns of the 64 TANTs. The blunted effect, the intensified effect, and the non-effect are indicated in blue, red, and gray blocks, respectively. Data are represented as mean ± standard deviation.

(B) The boxplots show the inter-population differences (Tibetans (in red) vs. Han (in blue)) of 6 representative TANTs. 1 to 2 km, 1000–2000 m; 3 to 4 km, 3000–4000 m. The details of the statistical assessment are provided in STAR methods. The asterisks in the frame refer to the significance of differences: ****, $p < 0.0001$; ***, $p < 0.001$, ns, not significant. For each boxplot, we draw a box from the first quartile to the third quartile. A vertical line goes through the box at the median. The whiskers go from each quartile to the minimum or maximum. The abbreviations of traits: BMI, body mass index; CREA, creatinine; TC, thorax circumference; TRIG, triglyceride; HR, heart rate; SBP, systolic blood pressure.

Uncover the novel adaptive traits in highland Tibetans

Cross-population comparison between Tibetan natives and Han migrants living at the same altitude provides a great opportunity to discover novel adaptive traits in Tibetans. We profiled 64 SDTs out of the 133 traits between Tibetans and Han in the HA cohort (Table S3). These SDTs were assumed to reflect the phenotypic adaptation in highland Tibetans,[11,15] and we therefore defined these traits as Tibetan adaptation-nominated traits (TANTs). Among the 64 identified TANTs, 45 of them (account for 70%) were previously unknown adaptive traits in Tibetans (Figure 3A; Table S3), in addition to the reported in Tibetans (such as the relatively lower hemoglobin level and the lower pulmonary pressure).[16–18]

Among the 45 newly identified TANTs, changes in spleen, lipid, kidney, and eye were worth noting (Figure S3). For example: (1) Tibetans have a thinner spleen than Han Chinese (spleen thickness, ST; −3.5%, $p = 9.2 \times 10^{-3}$), and this complies with the known mild proliferation of red cells induced by hypoxia in Tibetans that protects them from polycythemia[7,19] (Figure 3A). (2) Tibetans show a higher high-density lipoprotein (HDL; +5.0%, $p = 2.3 \times 10^{-7}$) without difference of total cholesterol (TCH; −0.2%, $p = 1$), implying that Tibetans might have acquired the ability of maintaining a healthy blood lipid metabolism (Figure 3A). (3) Tibetans have lower levels of blood uric acid (UA; −8.8%, $p = 3.3 \times 10^{-26}$) and urea nitrogen (BUN; −7.7%, $p = 3.5 \times 10^{-10}$), an indication of a better renal function (Figure 3A). (4) Tibetans have a lower central cornea thickness (CCTOS; −6.6%, $p = 1.9 \times 10^{-6}$, and CCTOD; −6.1%, $p = 1.5 \times 10^{-5}$), a lower spherical equivalent (SEOS; −53.6%, $p = 0.02$, and SEOD; −58.1%, $p = 1.0 \times 10^{-4}$), and a lower lens thickness (LTOS; −6.8%, $p = 2.0 \times 10^{-4}$, and LTOD; −6.9%, $p = 3.3 \times 10^{-4}$) of the eyes, presumably a physiological adaptation of eyes to strong ultraviolet radiation at altitude (Figure 3A).

Next, we intend to dissect the adaptive patterns of the 64 TANTs in highland Tibetans (STAR methods). Previous studies have reported blunted effects on blood hemoglobin and nitric oxide to protect Tibetans from hypoxia-induced polycythemia and peroxynitrite toxicosis.[19,20] We define "blunted effect" when a TANT change significantly less in Tibetan natives than in Han migrants when from LA to HA (STAR methods). Among the 64 TANTs, 16 have blunted effects spanning eight organ systems (body, blood, liver, kidney, lipid, metabolism, heart, and gallbladder) (Figure 3A; Table S3). Taking the case of the body traits, it is known that hypoxia tends to restrict body growth and development at high altitude,[21] while Tibetans were "blunted" to these restrictions, and exhibit higher values of weight (WT; +3.7%, $p = 8.5 \times 10^{-8}$), BMI (+5.6%, $p = 3.1 \times 10^{-22}$), thorax circumference (TC; +1.0%, $p = 3.1 \times 10^{-10}$), and waist circumference (WC; +3.0%, $p = 1.5 \times 10^{-3}$) than Han Chinese (Figure 3B). In particular, the larger TC in Tibetans indicates a better thorax development, likely contributing to their better ventilation at altitude.[9] Besides, we observed a lower pulmonary pressure in Tibetans with smaller degree of hypoxic pulmonary vasoconstriction (RVPG, TRV_MAX, TSV, TAPSE) as previously reported,[9] an indication of a significant blunted effect in high-altitude Tibetans.

We also found the TANTs exhibiting "intensified effect" (19 traits) in Tibetans, where Tibetans change more strikingly with altitude than Han migrants (Figure 3A; Table S3). For example, the serum creatinine significantly decreases in both Tibetans and Han with the increase of altitude, an indication of change of glomerular filtration rate due to reduction of oxygen utilization and/or supply,[22,23] but the drop in Tibetans was much larger than that in Han (−24.0% in Tibetans ($p = 5.2 \times 10^{-60}$) versus −10.0% in Han ($p = 1.5 \times 10^{-16}$)) (Figure 3B). Another example is peripheral oxygen saturation (SpO2), and the SpO2 level dropped 8.0% in Tibetans ($p = 5.1 \times 10^{-47}$) from LA to HA, which is larger than that in Han (a 7.0% drop, $p = 6.8 \times 10^{-188}$). This may imply a relatively minor hypoxia-induced kidney damage in Tibetans by keeping a relatively high glomerular filtration rate at high-altitude environment.

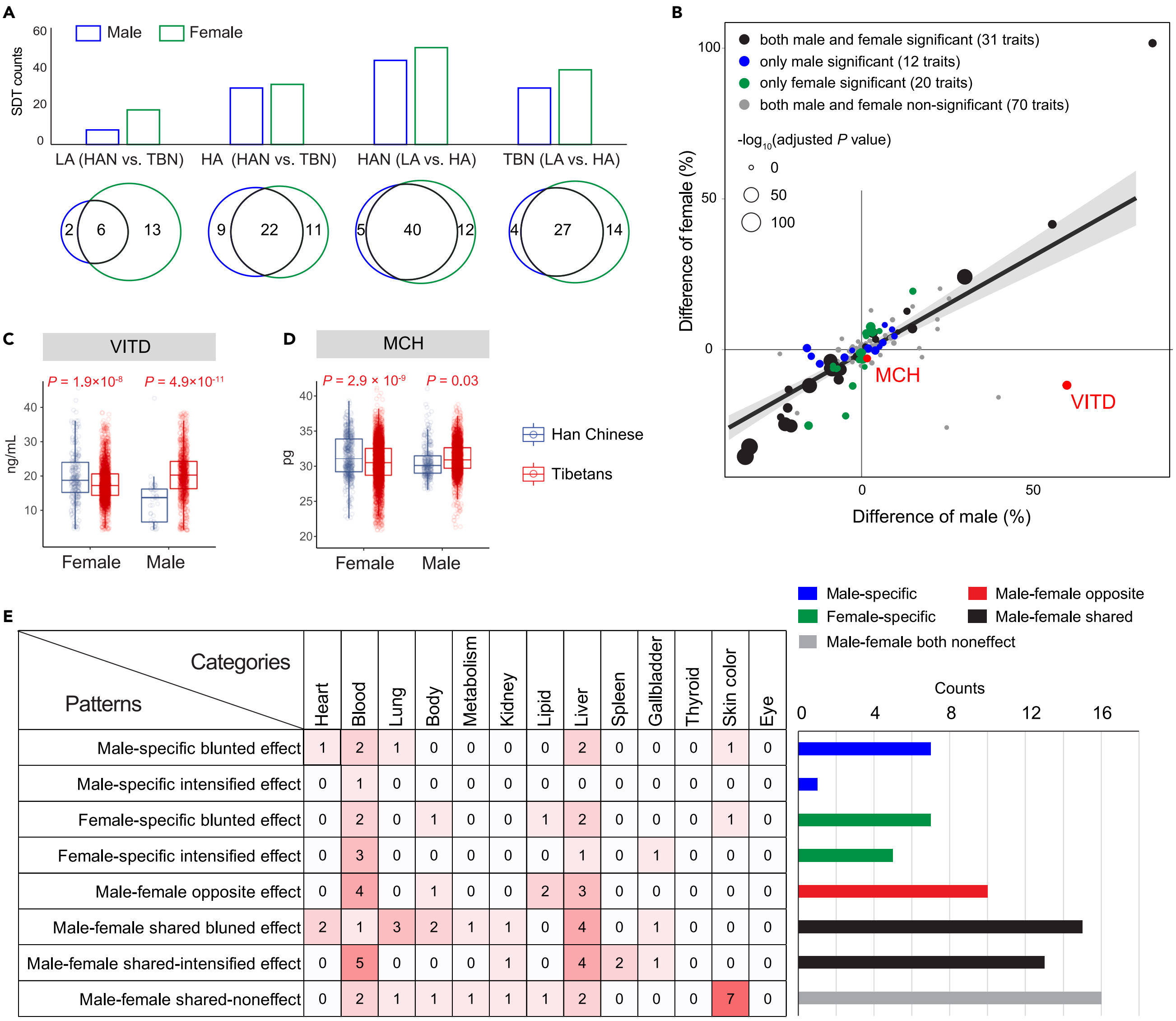
Additionally, we rechecked the adaptive patterns of the 6 previously reported traits in Tibetans, which were either controversial or lack of evidence for the proposed adaptation (Figure 3A; Table S3). There were three worth noting examples: (1) Tibetans have a lower triglyceride (TRIG) level (−23.9%, $p = 6.9 \times 10^{-60}$) and a higher fasting blood glucose (GLU; −9.4%, $p = 2.7 \times 10^{-37}$) than Han Chinese living at similar high altitude (Figure 3B), contradicting the reported higher TRIG in Tibetans as an adaptive fuel shift from fatty acid oxidation to glucose oxidation[24,25]; (2) Tibetans have lower serum folate level (FOLATE) than Han Chinese at high altitude (−35.8%, $p = 8.5 \times 10^{-141}$, Figure S2; Table S3), contradicting the previous proposal that FOLATE increasing is the adaptive strategy of Tibetans;[26] (3) Tibetans have a lower resting heart rate (HR) than Han at high altitude (−2.5%, $p = 2.3 \times 10^{-3}$), and more dramatically, HR significantly increases from LA to HA in Han (+10.0%, $p = 9.0 \times 10^{-24}$) but not in Tibetans (+6.0%, $p = 0.2$) (Figure 3B). This result rejects the previously proposed higher HR in Tibetans as an adaptive trait.[8,9] Instead, HR shows a blunted effect in Tibetans, and to improve oxygen delivery, Tibetans have acquired a higher systolic blood pressure to increase cardiac output (+3.6%, $p = 2.1 \times 10^{-14}$; Figure 3B; Table S3).

To test the developmental effects on high-altitude response of migrant populations,[11] based on the time they moved to high altitude, we classified the Han migrants into the acclimatization cohort (Han individuals who migrated to high altitude after adulthood, denoted as acHAN, $N = 851$) and the developmental cohort (Han individuals who were born and raised at high altitude, denoted as deHAN, $N = 271$) (STAR methods). For the 133 investigated traits, we observed a very high consistency between the mean value of each trait between acHAN and deHAN ($R = 0.99$, $p < 2.2 \times 10^{-16}$, Pearson correlation) (Figure S4; Table S4). Only 9 traits showed significant differences (evaluated by the ANCOVA test) between acHAN and deHAN, and four of them showed different pattern between the pairs of acHAN-TBN and deHAN-TBN, including MCV, RDWCV, PLT, and ALT. This suggests that corpuscular volume, size variance of red blood cell, platelets, and glutamate pyruvate transaminase of the Han migrants are subjected to developmental effect since they exhibited different patterns in acHAN and deHAN when compared to native Tibetans.

Sex-biased phenotypic differences between altitude adaptation and acclimatization

To assess sex differences between adaptation and acclimatization, we performed sex-separated phenotypic comparisons for the 4,246 males and 5,918 females (Table S5, STAR methods). There were 667 individuals with missing sex information, and they were excluded from the analysis. Markedly, we found a sex-biased pattern among SDTs between adaptation and acclimatization. In general, females have more cross-population and cross-altitude SDTs than males (Figures 4A and S5). We defined the sex-biased SDTs if a trait has significant difference between Tibetans and Han migrants only in one gender, and classified them into male- or female-specific SDTs accordingly. Among the 133 investigated traits, 32 were sex-biased SDTs, including 20 female-specific SDTs and 12 male-specific SDTs (Figure 4B; Table S5). The 20 female-specific SDTs cover metabolism (8 traits), body (4 traits), eye (4 traits), heart (2 traits), and blood (2 trait). By contrast, the male-specific SDTs cover blood (4 traits), metabolism (3 traits), skin-color traits (3 traits), and heart (2 traits) (Table S5). For example, BMI and CCTOS show significant differences between high-altitude Tibetan females and Han females ($p = 3.7 \times 10^{-24}$ and $p = 2.5 \times 10^{-4}$), but not in males ($p = 0.23$ and $p = 1$) (Figure S6). On the contrary, the free triiodothyronine level in blood and skin color of underarm were SDTs in males ($p = 5.3 \times 10^{-14}$ and $p = 7.7 \times 10^{-12}$), but not in females ($p = 1$ and $p = 0.31$) (Figure S6).

Notably, two TANTs show opposite patterns between females and males, including vitamin D (VITD) and mean corpuscular hemoglobin (MCH) (Figure 4B). VITD was the top trait with the largest between-sex difference, and Tibetan males have a significantly higher VITD level than Han males living at similar altitudes (+60.3%, $p = 4.9 \times 10^{-11}$), while the pattern was opposite in Tibetan females (−11.8%, $p = 1.9 \times 10^{-8}$)



Categories Patterns	Heart	Blood	Lung	Body	Metabolism	Kidney	Lipid	Liver	Spleen	Gallbladder	Thyroid	Skin color	Eye
Male-specific blunted effect	1	2	1	0	0	0	0	2	0	0	0	1	0
Male-specific intensified effect	0	1	0	0	0	0	0	0	0	0	0	0	0
Female-specific blunted effect	0	2	0	1	0	0	1	2	0	0	0	1	0
Female-specific intensified effect	0	3	0	0	0	0	0	1	0	1	0	0	0
Male-female opposite effect	0	4	0	1	0	0	2	3	0	0	0	0	0
Male-female shared bluned effect	2	1	3	2	1	1	0	4	0	1	0	0	0
Male-female shared-intensified effect	0	5	0	0	0	1	0	4	2	1	0	0	0
Male-female shared-noneffect	0	2	1	1	1	1	1	2	0	0	0	7	0

Figure 4. The sex-biased phenotypic patterns in altitude acclimatization and adaptation

(A) Counts of SDTs among the four pairwise comparisons in males (blue) and females (green), respectively. The Venn plots show the intersections between males and females among different comparisons. LA (low altitude: 1000–2000 m); HA (high altitude: 3000–4000 m); TBN, Tibetans; HAN, Han Chinese.

(B) Comparisons of the high-altitude Tibetan-Han differences between males and females. The dotplots indicate the difference percentages (calculated by (Tibetan-Han)/Han) of the 133 investigated traits. The significance levels (evaluated by the adjusted p-value) are denoted by dot size, and the four categories of significance combinations in view of males and females are showed by four different colors. The Pearson correlation was used to evaluate the consistency of males and females.

(C and D) The boxplots show the cross-population comparisons of two traits (VITD: Vitamin D, and MCH: mean corpuscular hemoglobin) that display the opposite patterns in males and females.

(E) The statistics of adaptive pattern comparison in males and females. The heatmap (left panel) shows the trait counts of the sex-biased patterns among the 13 organ/system categories, and the histogram (right panel) shows the trait counts among the eight sex-biased patterns. The sex-specific, opposite, and shared patterns are indicated by different colors, respectively.

(Figure 4C). For MCH, Tibetan males show an intensified effect with a higher MCH level than Han males (+1.6%, $p = 0.03$), while Tibetan females show a blunted effect with a lower MCH level than Han females (−2.9%, $p = 2.9 \times 10^{-9}$) (Figure 4D).

Furthermore, we classified the adaptive patterns of traits according to the definition of "blunted" and "intensified" effect as mentioned previously. The adaptive pattern was characterized as "specific" if it is only present in one sex, characterized as "shared" if present in both sexes, and characterized as "opposite" if it was different between males and females. We identified 30 traits with sex-specific adaptive patterns, including 7 male-specific and 7 female-specific traits showing blunted effects, and 1 male-specific and 5 female-specific traits showing

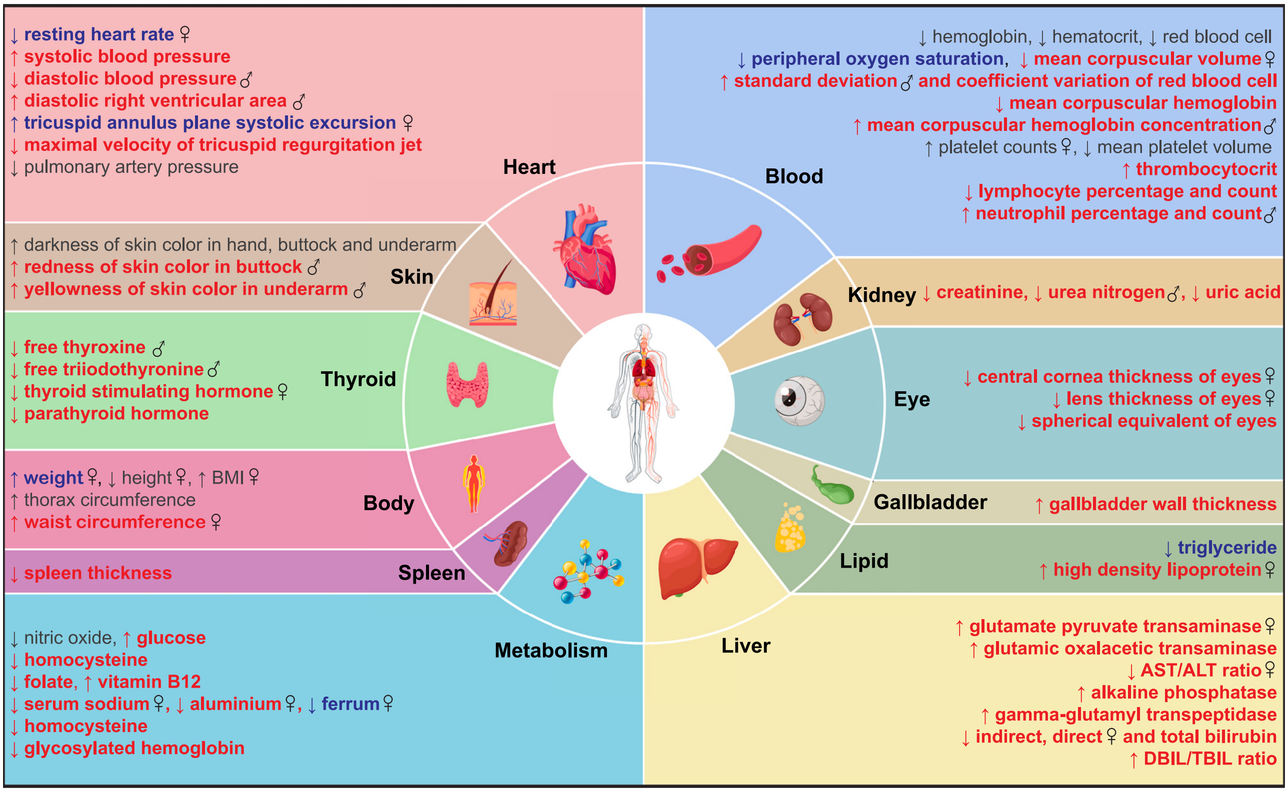


Figure 5. Atlas of the adaptive phenotypes in native Tibetans

The arrow directions refer to the Tibetan adaptive pattern of the analyzed phenotypes. The upward arrows indicate greater/higher values in Tibetans compared to the Han Chinese migrants at similar high altitudes, while the downward arrows indicate the opposite. The newly identified and re-checked adaptive traits of Tibetans are highlighted by bold font in red and blue, respectively. The male-specific and female-specific TANTs are denoted by ♂ and ♀, respectively, and the other TANTs are the shared TANTs between males and females. The 133 traits are classified into 13 organ systems.

intensified effects, an indication of a more prevalence of intensified effect in females than in males (Figure 4E; Table S5).In view of categories, the blood- and liver-associated traits were the most enriched categories in the sex-specific adaptive patterns, and females show adaptation in heart and lung traits, but males show adaptation in body and lipid traits (Figure 4E). Notably, 10 traits involving 5 categories show opposite adaptive patterns between females and males. For example, the Tibetan males have a less increased hematocrit (HCT) than Han males from LA to HA (+3.3% versus +10.0%, a typical blunted effect), but in females, Tibetans have more increase of HCT than Han from LA to HA (+10.1% versus +7.5%, a typical intensified effect) (Table S5).

Altogether, these results suggest that physiologically, there are sex-biased differences between altitude adaptation and acclimatization, underscoring the importance of sex-separated analysis in phenotypic profiling, which was often overlooked in the previous studies of highland populations.

DISCUSSION

The adaptation of Tibetans to high-altitude environments provides a real-time "experiment of nature" to understand the key evolutionary question of how the human body is shaped by natural selection. In this study, by measuring 133 traits in 10,831 highlanders, we systematically compared phenotypic similarities and divergences between altitude adaptation and acclimatization, and constructed an atlas of the adaptive phenotypes of native Tibetans at high altitude. In general, there were 12 body/organ systems showing adaptive phenotypic changes in Tibetans, including body, blood, heart, metabolism, kidney, lipid, liver, eye, spleen, thyroid, gallbladder, and skin (Figure 5; Table S6). This is expected considering oxygen delivery may affect the entire body though previous phenotypic studies were mainly focused on blood and cardiopulmonary systems.

We substantially expanded the spectrum of adaptive phenotypes in Tibetans by identifying 45 novel adaptive traits, especially covering internal organ states, metabolism, eye morphology, and skin pigmentation. Our data make it possible to view the global pattern of phenotypic adaptation, a multi-system adjustment likely resulting from the advantageous genetic variants enriched in Tibetans. In particular, these phenotypes on the previously ignored body/organ systems are highly informative, revealing several newly identified patterns. For example, as a blood filter, the spleen plays an important role regarding the metabolism of red blood cell and hemoglobin.[27] However, the spleen was not examined in indigenous highlanders, and only one study reported an increased spleen size in the diving population (Bajau) from Indonesia so that they create a larger reservoir of oxygenated red blood cells.[28] In contrast to the increased spleen size for adapting to acute hypoxia in Bajau, the spleen size of Tibetans is smaller than the Han Chinese migrants, suggesting a different strategy in the adjustments to the long-lasting hypoxia at altitude (Figures 3A and S3). Another example is the eye adaptation in Tibetans. We observed a lower central cornea thickness (CCT) in Tibetans than in Han Chinese (Figures 3A and S3). It was reported that CCT would become thicker when lowlanders move to high altitude,[29] suggesting that a lower CCT in Tibetans might be an adaptive physiological response to avoid eye damage from hypobaric hypoxia and/or high ultraviolet radiation.[30]

At the same time, we clarified the previously reported false adaptive patterns. For instance, the previous studies concluded that a higher serum TRIG level was adaptive for Tibetans, and they claimed such pattern is an adaptation by fuel shift from low-effective fatty acid oxidation to high-effective glucose oxidation/glycolysis under hypoxia.[24,25] However, these studies (including most of the published Tibetan studies) were lack of proper control populations, i.e., lowland migrants (e.g., Han Chinese) living at the same high altitude. We showed that, although the TRIG level in Han had a significant increase from low altitude to high altitude, the level in Tibetans had no difference (Table S2). Consequently, Tibetans display a relatively lower TRIG level at high altitude (Figure 3), suggesting fatty acid oxidation is a preferred fueling approach at high altitude. Conversely, the relatively higher TRIG in Han Chinese migrants is likely a reflection of tissue hypoxia when oxygen and ATP are insufficient, and TRIG cannot be efficiently consumed and/or stored in tissues.

Besides the previously reported blunted effect of blood hemoglobin and nitric oxide,[19,20] we also see similar blunted effects of many other traits, such as weight, resting heart rate, and gallbladder wall thickness, indicating that tuning down the sensitivity to high-altitude stresses (especially hypoxia) seems to be a key strategy adopted by Tibetans (Figure 3). For example, the blunted resting heart rate in Tibetans at high altitude might relate to the higher cardiac output, which may protect Tibetans from developing hypoxic pulmonary hypertension usually seen in lowlanders living at high altitude and the highland Andeans.[9] Such adaptive pattern has been observed in other long-resident species, e.g., the yak at high altitude,[31] suggesting the blunted effect might be a general strategy for the high-altitude adaptation. On the other hand, Tibetans also exhibited intensified effects, for example, the serum creatinine and peripheral oxygen saturation level etc. (Figure 3). These results demonstrate that the intensified effect is also an important adaptive mechanism for Tibetans. Future functional experiments are warranted to delineate the underlying genetic mechanisms of the blunted and intensified effects in Tibetans.

It was reported that the developmental effects might play an important role in high-altitude response of migrant populations.[11,14,32] Instead of genetic adaptation of native Tibetans, the migrants moving to high altitude at different phases of their life time can be recognized as physiological acclimatization (i.e., changes in organ components over days), which is different from the developmental effects (i.e., changes during the period of growth and development). Here, we investigated the developmental effects of 133 traits in the Han migrants, and found only 9 traits showed different patterns between high-altitude acclimatized Han and high-altitude developed Han. Our results suggest that the developmental effect may have influence on blood and metabolism traits in the high-altitude migrants, and in these indexes, the Han migrants who were born and raised at high altitude were more similar to native Tibetans than the Han migrants moving to high altitude during adulthood.

Another unexpected discovery was the pattern of sex-biased adaptation. Our results show that females have more cross-population and cross-altitude SDTs than males, suggesting that there might be a sex difference in high-altitude adaptation. This proposal is in line with several reports showing a better capacity of women/female mammals for hypoxic adaptation.[33–35] Also, this notion is supported by our observation of a more serious BW (birth weight) reduction and a higher prevalence of low BW in newborn males than females at high altitude.[36] Taken together, we speculate that sex difference might be a common phenomenon in high-altitude adaptation, reflected from both fetal development and adult survival. It is known that sex hormone is responsible for sex differences in stress response, such as hypoxic ventilation.[35] Whether the observed sex difference in high-altitude adaptation involves sex hormone is yet to be tested.

Taken together, we provided a comprehensive phenotypic atlas of high-altitude acclimatization and adaptation based on deep phenotyping of 11,880 highlanders. We characterized similar and different functional changes between adaptation and acclimatization, and established a landscape of adaptive phenotypes of indigenous Tibetans, including 45 newly identified Tibetan adaptation-nominated traits. We also observed a sex-biased pattern between altitude acclimatization and adaptation. The generated atlas of phenotypic landscape will significantly advance our understanding of human acclimatization/adaptation to high-altitude environments, which are informative for future medical and physiological studies.

Limitations of the study

There are limitations in this study. First, it should be noted that there are potential confounding factors that may affect the observed patterns of adaptive phenotypes. For example, the diet habits can affect some of the metabolic traits, which is difficult to completely rule out in our phenotypic profiling, despite all subjects fasted before the measurements. Besides, we observed a group of indexes indicating more liver damage in Tibetans than Han, which might be caused by the higher inherent hepatitis prevalence in Tibetan populations.[37] Although we have filtered out those individuals with diagnosed hepatitis, the potential liver effect caused by carriers of hepatitis B virus cannot be completely ruled out. Consequently, the proposed 45 TANTs in Tibetans might not be all adaptive, which calls for further detailed epidemiological surveys and genetic evidence.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107677.

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AUTHOR CONTRIBUTIONS

B.S., Y.H., and X.Q. designed the study. X.Q., W.Z., Y.G., C.C., Y.H., T.Y., H.Z., Ouzhuluobu, K.L., Z.Y., T.W., Z.B.J., J.Q., J.Y., and L.F. collected the samples and phenotypic data. Y.H. performed statistical analysis and made figures and tables. B.S. supervised the project; and Y.H. and B.S. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood samples from 9,948 Tibetans	This study	N/A
Blood samples from 2,456 Han Chinese	This study	N/A
Software and algorithms		
Code for statistical analysis	GitHub repository	https://github.com/heyaoxi/10KTibetan_Phenotype_analysis
aov	R	https://www.r-project.org/
lm	R	https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact: Bing Su (sub@mail.kiz.ac.cn).

Materials availability

This study did not generate new reagents.

Data and code availability

- All data generated in this study are available from the corresponding authors upon request.
- All original code has been deposited at GitHub. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this study is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The protocol of this study was reviewed and approved by the Internal Review Board of Kunming Institute of Zoology, Chinese Academy of Sciences (Approval ID: SMKX-20160311–45) and the research scheme is in accordance with the Regulations of the People’s Republic of China on the Administration of Human Genetic Resources. All participants signed the informed consent. To make sure the local native Tibetans can fully understand the content of the written consent, it was prepared in two language versions, Chinese and in Tibetan, and oral interpretation was provided for those who could not read the written consent.

We collected phenotypic traits and blood samples from 12,404 subjects (9,948 Tibetans and 2,456 Han Chinese) who lived in 13 different counties of the Qinghai–Tibetan Plateau, China, including 4,246 males and 5,918 females (the sex information of 667 individuals were missing). The age of study participant range from 7 to 93 years old. Considering sex and age being associated with the investigated traits (Table S5), we excluded individuals with missing sex information and only included those adult individuals (age range:18 years $\leq$ age $\leq$70 years). The high-altitude cohort including 9,825 Tibetans and 2,055 Han Chinese who living at elevation from 3,560m to 3,970m. The low-altitude cohort including 123 Tibetans and 401 Han Chinese living at elevation from 1,120m to 1,890m above sea level. The Tibetans are native residents at the sampled locations (they were born and grew up at the sampled locations), and the Han Chinese immigrant populations were sampled at the correspondent locations, and they had lived there for at least 1 year (Figure 1).

METHOD DETAILS

Phenotype measurements

A total of 133 phenotypic traits of 13 body/organ systems were measured, including heart, lung, blood, metabolism, eye, skin, liver, kidney, body, spleen, gallbladder, thyroid and lipid. All venous blood (5 mL from each individual) was collected after the subjects fasted overnight and the details of the measurement methods were described in Table S7.

Phenotype data analysis

For cross-population analysis, univariate comparisons of the average of each trait between Tibetans and Han Chinese of high-altitude cohort (elevation = 3000m–4000m) (or of low-altitude cohort (1000m–2000m)) were made by using the ANCOVA test in R *aov* function with sex, age and altitude as covariates: *aov (Trait ~ Ethnicity + sex + age + altitude)*. For analysis of eye traits, the education degree, frequency of reading, computer using, sunglass/sunhat using were also considered as covariates in the model.

For cross-altitude analysis, univariate comparisons of the average of each trait between low-altitude cohort (1000m–2000m) and high-altitude cohort (elevation = 3000m–4000m) in Tibetans (or in Han Chinese) were made by using the ANCOVA test in R *aov* function with sex and age as covariates: *aov (Trait ~ Altitude + sex + age)*.

For the sex-biased analysis, males and females were analyzed separately with age as covariates: *aov (Trait ~ Ethnicity + age)* for cross-population analysis, and *aov (Trait ~ Altitude + age)* for cross-altitude analysis.

The adjusted average (*adj.avg*) value of trait from each group was calculated using the *effects* package of R. All *p* values involving multiple comparisons in this study were adjusted by the Bonferroni test. A trait was defined as a significantly differential trait (SDT) when the adjusted p-value is smaller than 0.05.

The difference percentages of cross-population (Tibetans vs. Han) at HA were calculated by the formula: (Tibetan *adj.avg* at HA – Han *adj.avg* at HA)/(Han *adj.avg* at HA) * 100; LA is also the same. The difference percentages cross-altitude (LA vs. HA) in Tibetans were calculated by the formula: (Tibetan *adj.avg* at HA – Tibetan *adj.avg* at LA)/(Tibetan *adj.avg* at LA) * 100; Han Chinese is also the same. For the difference percentages between Tibetan changes from LA to HA and Han change from LA to HA: [Tibetans (*adj.avg* at HA – *adj.avg* at LA) - Han (*adj.avg* at HA – *adj.avg* at LA)]/Han (*adj.avg* at HA – *adj.avg* at LA) *100.

Determination of independent traits

The correlation of each trait pair was calculated using a covariate-adjusted regression linear model using the Tibetan data, by *lm* function of R language with age and sex as covariates: *lm (formula = Trait ~ Altitude + age + sex)*. Then, we determinated the cutoff of correlation coefficient R, so that how large R can be regard as correlation between two traits, by generating the distribution of correlation coefficient R of all trait pairs. The 5% cutoff of each side of the distribution was taken as correlation cutoff, namely, $R = -0.33$ and $R = 0.53$ (Figure S7). Any trait pair with $R > 0.53$ in positive correlation or $R < -0.33$ in negative correlation will be regard as correlated pair and one of them was excluded from the 133 traits. Taking blood traits as example, after excluding the correlated pairs (Figure S7), the 21 traits reduced to 11 independent traits. Totally, among the 133 traits, we got 87 independent traits.

Evaluation of the adaptive patterns of phenotypes

We evaluated the adaptive patterns of TANTs by the following criteria: 1) the change direction of Tibetans from low altitude to high altitude is same with the change in Han; 2) a TANT is defined as "blunted effect" when Tibetans change (LA vs. HA) less than Han; 3) a TANT is defined as "intensified effect" when Tibetans change (LA vs. HA) more than Han; 4) the other TANTs were classified into the category of "non-effect" (Table S3).

Evaluation of developmental effect on the Han migrants

We classified the Han migrants based on the time they lived at high altitude as previously reported[11,14]: the acclimatization cohort refers to the Han individuals who migrated to high altitude during adulthood (defined by age >18); and the developmental cohort refers to the Han individuals who were born and raised at high altitude. In total, 851 Han belong to the acclimatization cohort (acHAN) and 271 Han to the developmental cohort (deHAN), and they lived at the same high-altitude region (3000m–4000m).

QUANTIFICATION AND STATISTICAL ANALYSIS

Quality control

For 12,404 subjects, we filtered the samples based on the following criteria: (1) ancestry identification: the ethnic identity of Tibetan and Han Chinese was confirmed by self-claims, identity card and report of the first language learned, and only those individuals without mix-ancestry within three generations were included; (2) nonsmoking and nondrinking (by self-report); (3) 18 years ≤ age ≤70 years; (4) hepatitis B/C negative; (5) no family history of known genetic diseases. In total, 1,573 individuals were filtered out, and 10,831 individuals were included in the downstream analyses.

Statistical analysis

Univariate comparisons of the average of each trait between Tibetans and Han Chinese were made by using the ANCOVA test of *aov* function in R, and the correlation of each trait pair was calculated using a covariate-adjusted regression linear model using *lm* function of R (see method details).