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Genetic evidence of a recent Tibetan ancestry to Sherpas in the Himalayan region

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Sherpas living around the Himalayas are renowned as high-altitude mountain climbers but when and where the Sherpa people originated from remains contentious. In this study, we collected DNA samples from 582 Sherpas living in Nepal and Tibet Autonomous Region of China to study the genetic diversity of both their maternal (mitochondrial DNA) and paternal (Y chromosome) lineages. Analysis showed that Sherpas share most of their paternal and maternal lineages with indigenous Tibetans, representing a recently derived sub-lineage. The estimated ages of two Sherpa-specific mtDNA sub-haplogroups (C4a3b1 and A15c1) indicate a shallow genetic divergence between Sherpas and Tibetans less than 1,500 years ago. These findings reject the previous theory that Sherpa and Han Chinese served as dual ancestral populations of Tibetans, and conversely suggest that Tibetans are the ancestral populations of the Sherpas, whose adaptive traits for high altitude were recently inherited from their ancestors in Tibet.

Following the groundbreaking ascent of Mount Everest by Tenzing Norgay and Sir Edmund Hillary, the Sherpa people gained such cachet that their name became vernacular shorthand and pop culture reference for much of the twentieth century. Curiously, despite the hypoxic conditions of their highland homes in the Khumbu region of Nepal and smaller settlements along the Sino-Nepalese border in the Tibet Autonomous Region of China in Dingjie County and Zhangmu Town, Sherpas seem to cope well with the environments and avoid altitude sickness. This heightened adaptation to high altitude hypoxic environments seems to suggest that the Sherpas have been living in the region for a relatively long time and acquired an inherited adaptation. Unfortunately, archaeological evidence from the Himalayas that might verify this hypothesis is quite limited. The existing archaeological data suggests that the first peoples arrived at the Tibetan plateau as early as 30,000 years ago and were later followed by successive migrations from different times and places, ultimately creating a complex mosaic of population history at the Himalayas¹. Specifically, some stone tools in western Tibet were dated to 25,000 to 20,000 years ago and were claimed to be similar to other stone tools excavated in Nepal, hinting at a northward migration into the Tibetan plateau from the southern Himalayas^{1,2}.

Some genetic evidence also supports the Nepal-Tibet migrations, though in different patterns. A recent genetic study went so far as to posit that Sherpas and Han Chinese were the two ancestral populations of modern Tibetans, and consequently the genetic adaptation to high altitude hypoxia seen among Tibetans was likely inherited from the ancestral Sherpas³. Conversely, a recent study of mtDNA

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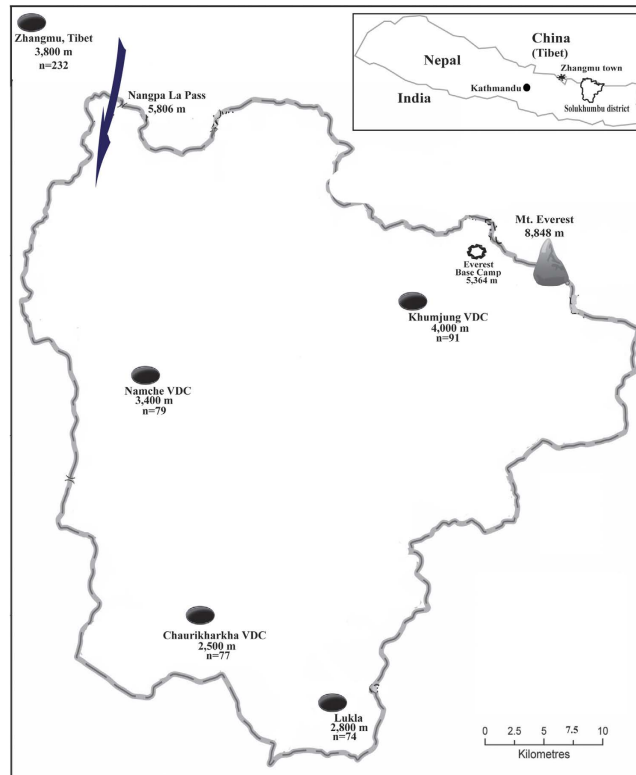


Figure 1. Sampling locations of Sherpa populations in Nepal and Tibet. The altitudes of the locations (dark spots) range from 2,500 m–4,000 m. The proposed Tibet–Nepal migratory route of the Sherpa ancestors through Nangpa La Pass is indicated with an arrow. The map was created using Surfer10 (Golden Software Inc., Golden, USA), and the figure was generated using Microsoft Powerpoint 2011 (Microsoft Corporation, USA).

diversity on a small Sherpa sample from Zhangmu Town in Tibet reported two Sherpa-specific haplotypes with relatively young ages (<1,500 years), suggesting a Tibet into Nepal migration granting the Sherpas' well-known adaptation at high altitudes⁴. The disparity in these two views reflects a fundamental divide in the evolution of adaptive features among Sherpas, holding to either a short (<1500 years) or long (30,000 years) duration of colonization at high altitude. Complicating this controversy is the limited genetic studies of populations from the southern Himalayas, leaving the answer of when and where the Sherpa people originated and settled at the high altitude regions of Nepal unclear.

In the present study, we analyzed Y-chromosome and mtDNA diversity of 582 unrelated Sherpa samples collected from different places in both Nepal and Tibet (Fig. 1). We genotyped the Y chromosomal single nucleotide polymorphisms (Y-SNPs) and Y short tandem repeats (Y-STRs) from 277 male Sherpa samples. We also sequenced the complete mtDNA genomes of 89 Sherpa individuals and genotyped the remaining samples based on the control region (HVS-I and HVS-II) and partial coding regions of mtDNA. Data from both the paternal (Y chromosome) and the maternal (mtDNA) markers indicates that the Sherpa people are a recently derived sub-lineage of Tibetans, supporting a short colonization and settlement in the southern Himalayas.

Results

Y-chromosomal diversity in Sherpa population. In total, DNA samples of 582 unrelated Sherpa individuals representing two geographic populations from Nepal (350 individuals) and Tibet (232) were collected along with samples of 90 non-Sherpa Nepalese from Solukhumbu district of Nepal (Fig. 1). Using the current phylogeny of the human Y-chromosomes, we assigned the samples from 277 Sherpa males into four major Y-chromosomal haplogroups including D-M174 (44.04%), O-M175 (27.08%), F-M89 (9.75%), and K*M9 (7.22%) (Fig. 2A). Of these groups, D-M174 and O-M175 are also the two dominant Y-haplogroups in Tibetans (52.84% and 33.13% respectively)⁵, suggesting a close paternal relation between Sherpas and Tibetans. Likewise, we observed several rare (0.36–3.61%) haplogroups in Sherpas (G-M201, J-M304, M-P256, N-M231, P-M45, Q-M242 and R-M207) that are also seen among Tibetans in similarly low frequencies (Fig. 2A). Due to the similarities in the distributions of Y-haplogroups between Sherpas from Nepal and those from Tibet, the data were merged together for further analyses.

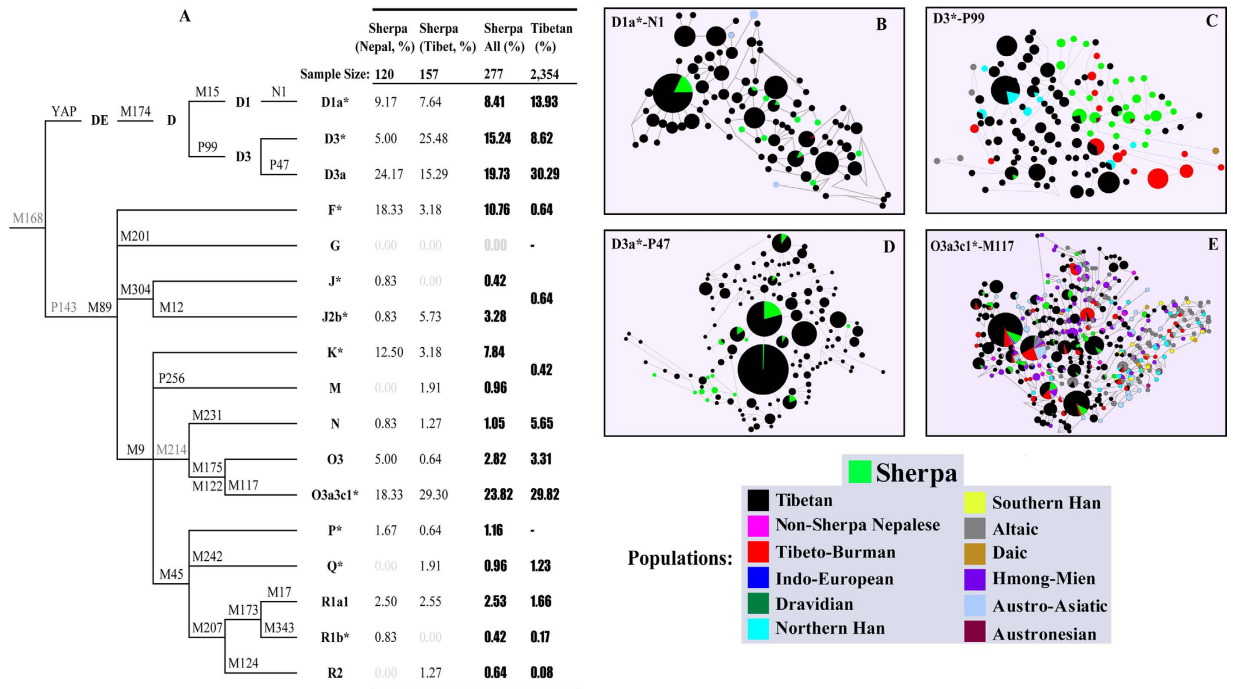


Figure 2. Comparison of Y chromosome diversity among Sherpas, Tibetans and other Asian populations.

(A) Phylogenetic tree of the Y chromosome haplogroups and their frequency distributions in Sherpas and Tibetans. (B–E) The Y-STR networks of the four major haplogroups showing the distributions of STR haplotypes in Sherpas and other Asian populations. Populations were labeled with different colors based on their belonged language families.

Comparing Sherpas, Tibetans, and Han Chinese showed that the D-M174 is the predominant haplogroup in Sherpas (43.38%) and prevalent in Tibetans (52.84%)⁵, but rare among both Han Chinese (1.4–6.51%)^{6,7} and other Asian populations (0.02–0.07%)⁸, aside from Japanese (34.7%) who possesses a distinct D-M174 lineage highly diverged from those in Tibetans and other Asian populations^{9,10}. Among Tibetans there are five D-M174 sub-haplogroups (D*-M174, 0.08%; D1*-M15, 1.40%; D1a-N1, 13.93%; D3*-P99, 8.62%; D3a-P47, 30.29%), three of which are also present among Sherpas (D1a-N1, 8.41%; D3*-P99, 15.24%; D3a-P47, 19.73%; see Fig. 2A). To explore the structure of the D-M174 sub-haplogroups, we constructed the Y-STR network of these sub-lineages and observed that the Y-STR haplotypes among Sherpas represent a subset of those found in Tibetans, implying that the paternal lineages of Sherpas were likely derived from those in Tibetans (Fig. 2B–D). Moreover, there were Sherpa-specific Y-STR haplotypes under the D3*-P99 sub-haplogroup closely linked with those in Tibetans (Fig. 2C), implying a shallow divergence between Sherpas and Tibetans at this paternal lineage.

A similar pattern of Y-STR network was also seen among the second most common haplogroup O3a3c1*-M117 in Sherpas (23.82%), which is also present in Tibetans (29.82%), Han Chinese (9.6–16.3%) and many other East Asian and Southeast Asian populations (5.5–16%)⁵, but Sherpas shared most of their Y-STR haplotypes of O3a3c1-M117 with Tibetans (Fig. 2E).

Taken together, D-M174 and O-M175 account for 71.12% of the paternal lineages in Sherpas, bolstering the case for Tibetans as the ancestral population of Sherpas. Likewise, the relative rarity of other Y haplogroups (<10%) among Sherpas (supplementary Figure S1) were also rare among Tibetans, and mostly absent in other East Asian populations. Most of these haplogroups are prevalent in India (F*-M89, J2b*-M12 and R1a1-M17) and Island Southeast Asia (K*-M9), suggesting either shared ancient Y-chromosome lineages or limited recent admixture of Sherpas with surrounding populations.

Mitochondrial DNA diversity in Sherpas. Classification of mtDNA haplogroups of 582 Sherpa samples yielded several major haplogroups including A (27.15%), M9a (24.23%), C4a (20.96%), M70 (7.22%) and D (5.84%) (Table 1), which together cover 85.40% of the tested Sherpas. Among Tibetans these same groups A (14.63%), M9a (22.48%) and D (16.53%) also constituted the major haplogroups⁵. M9a and its four sub-haplogroups (M9a1a, M9a1a2, M9a1b1 and M9a1a1c1b1a) are widely distributed in East Asia and Southeast Asia^{11,12}, though M9a1a1c1b1a is quite rare among most Asian populations but among Sherpas is the most common (58.16% of the total M9a individuals) and among Tibetans highly prevalent (58.06% of the total M9a individuals)¹². The shared prevalence of M9a1a1c1b1a in Sherpas and Tibetans suggests a close maternal relationship between them. After constructing a network using 36 complete mtDNA genome sequences (34 previously published and two of present study) from 13

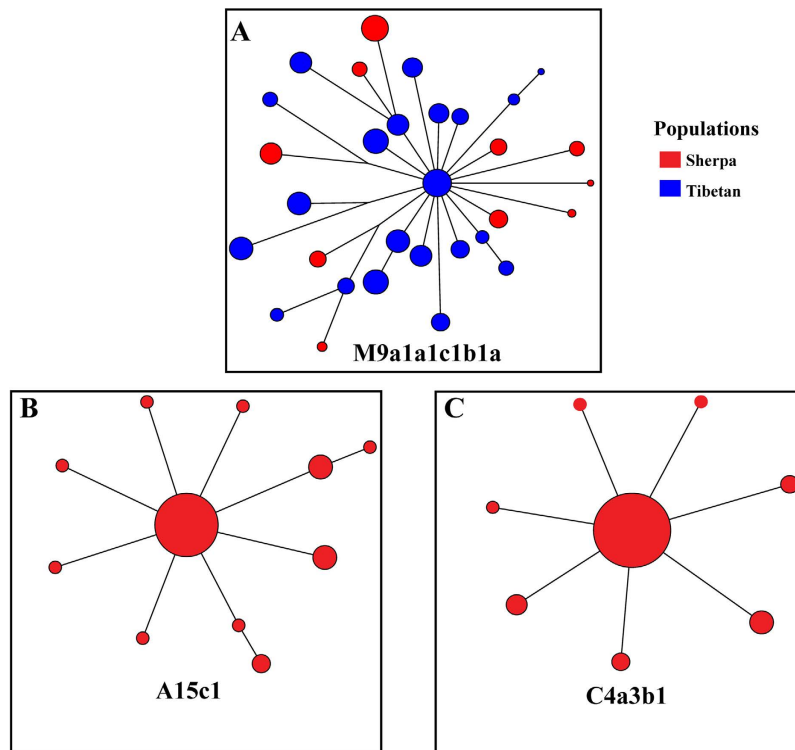


Figure 3. Networks of three mtDNA sub-haplogroups (A: M9a1a1c1b1a; B: A15c1; C: C4a3b1) among Sherpas and Tibetans. The star-like networks suggest recent population expansion. The complete mtDNA genome sequences were used to construct the networks.

Sherpas and 23 Tibetans belonging to M9a1a1c1b1a, we observed that all the Sherpa individuals lie at the tip branches, suggesting they likely derived from the Tibetan core haplotype (Fig. 3A). Interestingly, the star-like topology (*i.e.* a core haplotype surrounded by other haplotypes with only one or two mutation steps away from the core) suggests a relatively recent expansion of this lineage, consistent with a previous observation made on Tibetans⁵. The close genetic affinity between the two populations is further supported by Haplogroup D among Sherpas (5.84%), which was also widely found in Tibetans⁵. But more importantly M70—previously considered a Tibetan specific lineage^{13,14}—was also present among Sherpas (7.22%) (Table 1). Much like the observed pattern of the Y-haplogroups, the Sherpa mtDNA haplogroup composition bears strong similarity with that observed among Tibetans.

Interestingly, two Sherpa-specific sub-haplogroups were not detected in Tibetans or other Asian populations: A15c1 (17.27%), a sub-lineage under Haplogroup A15c, and C4a3b1 (21.82%), a sub-lineage under C4a3b⁴. These two Sherpa-specific sub-haplogroups together account for nearly 40% of the maternal lineages of Sherpas. Sequencing the entire mitochondrial genomes of 79 Sherpa individuals belonging to these two sub-haplogroups showed an interesting sequence variation pattern. Consistent with the previous report⁴, A15c1 defined by four mutations (T4216C, A9052G, T13111C and A15924G), and C4a3b1 by other four mutations (G3745A, 5899insC, C11155T, A13563G) only exist in Sherpas, but their mother haplogroups (A15c and C4a3b) are present in both Tibetans and Han Chinese^{15,16} (supplementary Figure S2). Further network analysis showed that A15c1 and C4a3b1 both form a star-like structure, denoting recent lineage expansion among Sherpas (Fig. 3B,C). We estimated the coalescence ages of A15c1 (1500 years ago) and C4a3b1 (940 years ago), both of which are similar with the previous estimates⁴ and consistent with the proposed recent expansion. The young ages of these two Sherpa-specific sub-haplogroups hint at a recent bottleneck during migration and latter population expansion of Sherpas, which fits our observation of a shallow divergence of the Y chromosome haplogroup D3*-P99 between Sherpas and Tibetans.

Of the other rare mtDNA haplogroups in Sherpas—Z (2.75%), F (2.58%), M13 (1.72%) and U (1.37%), as well as those with <1% frequencies (M3, M5, W, G, M10, H, M62, M11a, M38, M61 and M74)—a majority including G, Z, F, M62, M10, M11a, M13, and F have been seen among Tibetans. By contrast, the presence of the other haplogroups M5, M3, W, M38, M61, M74 and U among Sherpas may indicate minor gene flows from surrounding South Asian populations (see Table 1).

Genetic relationship of Sherpas with other Asian populations. We explored the genetic relationship between Sherpas and other Asian populations, and principal component analysis (PCA) of both mtDNA and Y chromosome haplotype frequencies displayed a close relationship between Sherpas and

Haplogroup frequency (%)				
Populations	Sherpa (Nepal)	Sherpa (Tibet)	Sherpa (all)	Tibetan ⁵
Sample Size	350	232	582	6,109
A	24.00	31.90	27.15	14.63
M9a	27.14	19.83	24.22	22.48
M8	28.00	17.24	23.71	7.71
M70	11.14	1.29	7.22	0.16
D	4.57	7.76	5.84	16.53
F	1.14	4.74	2.58	11.44
M13	0.57	3.45	1.72	4.22
U	0.29	3.02	1.37	1.65
M5	0.29	1.72	0.86	0.05
W	0.57	1.29	0.86	0.05
M3	0.29	1.72	0.86	0.00
G	0.57	0.86	0.69	8.22
M10	0.29	1.29	0.69	1.06
M62	0.00	1.29	0.52	2.35
M11a	0.29	0.86	0.52	0.79
H	0.00	1.29	0.52	0.26
M38	0.29	0.43	0.34	0.00
M61	0.29	0.00	0.17	0.75
M74	0.29	0.00	0.17	0.00
B	0.00	0.00	0.00	3.76
M*	0.00	0.00	0.00	0.79
TJ	0.00	0.00	0.00	0.72
R*	0.00	0.00	0.00	0.52
N*	0.00	0.00	0.00	0.47
M7	0.00	0.00	0.00	0.25
N10	0.00	0.00	0.00	0.25
M25	0.00	0.00	0.00	0.23
M20	0.00	0.00	0.00	0.20
N9a1	0.00	0.00	0.00	0.20
N11	0.00	0.00	0.00	0.18
M49	0.00	0.00	0.00	0.07
M12	0.00	0.00	0.00	0.02
Y	0.00	0.00	0.00	0.02
M12	0.00	0.00	0.00	0.02

Table 1. Distribution of mtDNA haplogroups among Sherpas and Tibetans.

Tibetans (Fig. 4A,B). In particular, the PCA map of the Y chromosome shows Sherpas mingled together with Tibetan populations and forming a separate cluster from other Asian populations (Fig. 4B). The PCA map of mtDNA further places the Sherpas relatively closer to Tibetans compared to other Asian populations but form a separate cluster, implying a shallow divergence from Tibetans likely due to the recent expansions of the two Sherpa-specific mtDNA sub-haplogroups (A15c1 and C4a3b1) (Fig. 4A). By contrast, non-Sherpa Nepalese are only distantly related to Sherpas, clustering with populations from the Indian subcontinent and East Asia, suggesting limited gene flows between Sherpas and non-Sherpas in the markedly small area of Nepal nestled between India and China.

Discussions

Our investigation of the Sherpas' origins via analysis of Y-chromosome and mtDNA diversity analysis supports a close genetic relationship between Sherpas and Tibetans. Furthermore, the detailed genetic diversity pattern revealed in the Y-STR network and the mtDNA phylogenetic trees indicate that Sherpas

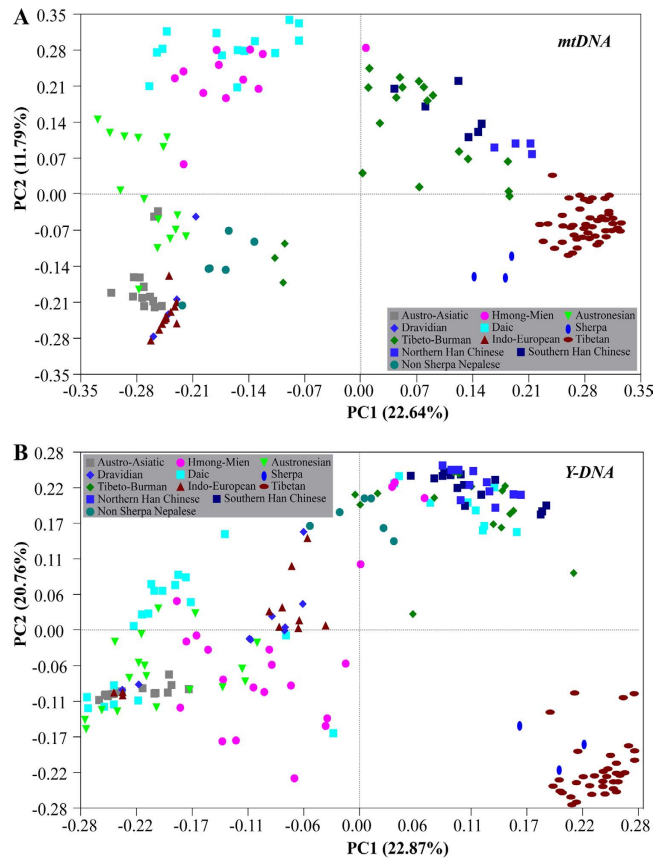


Figure 4. Maps of principal component analysis (PCA) based on mtDNA and Y-Chromosome haplogroup frequencies among Sherpas, Tibetans and other Asian populations.

represent a recently derived lineage from Tibetans dated to less than 1,500 years ago. Further BSP analysis sketches a general picture of a recent bottleneck of the Sherpa population size less than 2,000 years ago (supplementary Figure S3), consistent with the proposed recent migration of the Sherpa ancestors from Tibet. These genetic findings further bolster the established linguistic affiliation among the speakers of the Tibeto-Burman language subfamily within the Sino-Tibetan family (the other subfamily is Han). Collectively, these results concur with the previous hypothesis positing the migration of the Baric branch of the Tibeto-Burman languages from the Tibetan plateau to Nepal following their dispersal from the basin of Yellow River in China⁷.

Curiously though, a previous mtDNA study of Zhangmu Sherpa reported considerable (8%–17%) South Asian genetic component among Sherpas¹⁷, but our results only show it in a minor frequency (0.34%–2.53%). Likely, this negligible gene flow from the Indian subcontinent towards the Sherpa is due to the natural barrier of the Himalayas, that have effectively firewalled the populations in the mountainous, low-oxygen environments that comprise the Sherpa homeland^{5,18,19}.

Our observation of the large amount of East Asian genetic influence on the Nepalese population (Sherpas and non-Sherpas) suggests a recent unidirectional gene flow from East Asia to Nepal, not the opposite^{20,21}. By the same token, our present data does not support the putative ancestry of the Sherpas and Han Chinese as progenitors of Tibetans³, and instead strongly supports that the Tibetans are ancestors of the Sherpas. While two Sherpa-specific mtDNA sub-lineages were found during our study, both are young in origin (<1500 years ago), and originated rather recently from the mother haplogroups of Tibetans, in line with recent historical records over the last 500 years ago that documents Sherpa migrations from eastern Tibet to the barren lands of Nepal's Khumbu region by crossing the Nangpa La Pass (5,716m) in the Himalayan region (Fig. 1)²².

As for the Sherpa-specific sub-haplogroups, a previous mtDNA study⁴ proposed that the ND1 variants G3745A (C4a3b1) and T4216C (A15c1) may form the molecular basis of the Sherpas' adaptation to hypoxic high-altitude. After conducting a selection test by calculating the non-synonymous vs. synonymous substitution (Ka/Ks) ratio of ND1, we did not observe a signal of selection (Ka/Ks = 1.0, Nei-Gojobori model). We also conducted Tajima's D test using both the ND1 and HVS-I sequences, and no deviation from neutral expectation was detected (Tajima's D = -1.271 for ND1, $P > 0.1$; Tajima's D = -1.168 for HVS-I, $P > 0.1$), suggesting a neutral effect of these variants. Hence, the prevalence of the two Sherpa-specific mtDNA sub-lineages may better be explained by recent population expansion

as reflected by the star-like phylogeny and their tip positions in the mtDNA phylogenetic tree (supplementary Figure S1). Accordingly, we propose that the ND1 variants may not have any direct role in hypoxic adaptation among Sherpas; indeed, our results suggest that the ancestors of Sherpas were already high altitude dwellers in the Tibetan plateau before they migrated from Tibet rather recently, which by extension predicts the same set of genes responsible for high-altitude previously identified in Tibetans (e.g. EPAS1 and EGLN1)^{23,24} should also be present among the Sherpas. In essence, the genetic adaptation to high altitude in Sherpas were not likely to have developed locally in Nepal, but were instead inherited from their Tibetan ancestors who acquired the adaptive traits through their long stay (>18,000 years)²³ in the harsh environment of the highland Tibetan plateau.

Conclusion

Our mtDNA and Y-chromosome analyses of Sherpas indicate that Tibetans were the ancestral population of the Sherpas, and consequently that Sherpas likely acquired their high altitude adaptive features during their ancestors' long-stay on the Tibetan plateau before their more recent migration towards Nepal. This recent origin of Sherpa-specific mtDNA sub-lineages may then not play any direct role in hypoxic adaptation. Ultimately, these genetic analyses both clarify long-standing debate on the origin of the Sherpas but also prompt the possibility that similar molecular mechanism for high altitude adaptation are shared between both Sherpas and Tibetans.

Materials and Methods

Sample collection. Blood samples of 2–5 ml were collected from 582 Sherpa individuals (350 from four villages of the Khumbu region of Nepal and 232 from Zhangmu Town of Tibet) (Fig. 1). We also sampled 90 non-Sherpa Nepalese from Solukhumbu district of Nepal (the lowland region with elevation <2000 m) who speak Tibeto-Burman languages. Volunteers were asked to reveal the ethnic affinity of their parents and grand-parents in an oral interview, and only volunteers whose parents and grand-parents were reported to be Sherpas were included in this study. Sample collection was done randomly from individuals unrelated for at least three generations. All participants provided written informed consent prior to inclusion in the study. The protocols of this study were approved by the Internal Review Boards of the Kunming Institute of Zoology, Chinese Academy of Sciences and the Nepal Health Research Council, Kathmandu, Nepal. All methods were carried out in accordance with the approved guidelines.

Y-chromosomal genotyping. The Y-chromosomal SNP markers were genotyped using methods similar to those described in our previous studies^{10,25,26}, and paternal haplogroups were classified based on the up-to-date high-resolution Y-chromosomal phylogenetic tree²⁷. The commonly used eight Y-chromosomal STR (short tandem repeat) markers (DYS19, DYS388, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393) were typed using fluorescence-labeled primers in an ABI 3130XL Genetic Analyzer (Applied Bio systems, USA). The nomenclature of Y-chromosomal STRs followed the process proposed by Butler *et al.* (2002)²⁸.

Mitochondrial DNA sequencing and genotyping. The mtDNA HVS-I (range:16024–16466), HVS-II (range:65–417) and a coding region (range:10,220–10,610) were sequenced following the protocol described previously²⁹ and then we sequenced several other coding region sites for further confirmation used to define each individual within their respective mtDNA haplogroups. We then conducted amplicon sequencing of the entire mitochondrial genomes of 89 Sherpa samples using Illumina Miseq, including 79 individuals belonging to the two Sherpa-specific sub-haplogroups (32 A15c1 and 47 C4a3b1) and the other 10 individuals belonging to other haplogroups (5 M9a, 1 M70, 1 C4a1a1, 1 D4j1b and 1 A15c). The complete mtDNA was amplified via PCR of two overlapping fragments (9.3 kb and 9.2 kb)³⁰. Amplicons were analyzed by agarose gel electrophoresis, individually purified via resin purification (Promega), quantified on a Bioanalyzer (Agilent Technologies 2100 Bioanalyzer) and equimolarly pooled. The Nextera DNA Sample Preparation guide (15027987) was followed for pooled amplicons to create indexed, paired-end libraries that were later sequenced on an Illumina Miseq (2 × 300 base reads) using the manufacturer's protocol (Illumina, San Diego, CA). The resulting high quality sequence data from Miseq had an average coverage of 2000×. Sequence analysis was then done using mtDNA MSR Plug-In and the mtDNA Variant Analyzer provided by Illumina in Human mtDNA Genome Guide (15037958) for the Illumina Sequencing platform. (http://support.illumina.com/downloads/human_mtdna_genome_guide_15037958.html). The mtDNA variants were identified by comparing each sequence with the revised Cambridge Reference Sequence (rCRS)³¹, and haplogroups were assigned based on the mtDNA phylogenetic tree (Phylo Treemt Build 16,19 Feb 2014)³². Comparing a sample the variants produced by Miseq with those from Sanger sequencing confirmed that our MiSeq data were highly accurate and reliable.

Data analysis. The Sherpa mtDNA phylogenetic tree was constructed based on the available whole genome mtDNA sequences of 165 Sherpa samples, including 76 Sherpa samples from previous studies⁴ and 89 samples obtained in this study. The Phylo Tree mt Build 16³² was followed for constructing the phylogenetic tree with exclusion of several mutations (309.1C (C), 315.1C, AC indels at 515–522, 16182C, 16183C, 16193.1C (C) and 16519). The median-joining network was constructed using NETWORK

4.6.1.0 (Fluxus Engineering)³³ (Bandelt *et al.* 1999), and was constructed for the major haplogroups of both mtDNA and Y chromosome.

The coalescence times of the dominant mtDNA haplogroups were estimated using the ρ statistics³⁴, and standard errors were calculated following Saillard *et al.*³⁵. The complete mtDNA genome sequences were used for age estimations by excluding 309.1C (C), 315.1C, AC indels at 515–522, 16182C, 16183C, 16193.1C (C) and 16519). The mutation rate of 3,624 years per mutation was used for entire mtDNA genome to estimate the timeframe for the most recent common ancestor (TMRCA) of a haplogroup³⁶.

To observe the genetic relationships of Sherpas with other populations, we constructed PCA maps based on both mtDNA and Y-Chromosome haplogroup frequencies as described previously using MVSP3.13 (Kovach Computing Services, Anglesey, UK)³⁷. For the neutrality test, the ratio of non-synonymous vs. synonymous substitutions in the ND1 gene of Sherpas were analyzed using the Nei-Gojobori model³⁸ in the MEGA5 package³⁹. We also conducted Tajima's D test using DnaSP Version 5⁴⁰. We reconstructed the demographic history of Sherpas using BSP⁴¹ in BEAST 1.7.5⁴² using MCMC algorithms⁴³. The HVS-I (16038–16462) sequences of available 661 Sherpa individuals were used for generating BSP. A clock rate of 1.784×10^{-7} substitutions per site per year³⁶ was applied. MCMC sample was based on run of 5×10^8 steps with sampling every 5,000 steps, and the initial 5×10^7 were regarded as burn-in. The effective sample size values were in acceptable range (>100) for all the runs. Tracer 1.5 (<http://tree.bio.ed.ac.uk/software/tracer>) in the MCMC Trace analysis packages was used to visualize the BSP.

References

- Aldenderfer, M. Peopling the Tibetan plateau: insights from archaeology. *High Alt. Med. Biol.* **12**, 141–147 (2011).
- Qiu, J. Who are the Tibetans? *Science* **347**, 708–711 (2015).
- Jeong, C. *et al.* Admixture facilitates genetic adaptations to high altitude in Tibet. *Nat. Comm.* **5** (2014).
- Kang, L. *et al.* mtDNA lineage expansions in Sherpa population suggest adaptive evolution in Tibetan highlands. *Mol. Biol. Evol.* **30**, 2579–2587 (2013).
- Qi, X. *et al.* Genetic evidence of paleolithic colonization and neolithic expansion of modern humans on the tibetan plateau. *Mol. Biol. Evol.* **30**, 1761–1778 (2013).
- Su, B. *et al.* Y-Chromosome evidence for a northward migration of modern humans into Eastern Asia during the last Ice Age. *Am. J. Hum. Genet.* **65**, 1718–1724 (1999).
- Su, B. *et al.* Y chromosome haplotypes reveal prehistorical migrations to the Himalayas. *Hum. Genet.* **107**, 582–590 (2000).
- Wells, R. S. *et al.* The Eurasian heartland: a continental perspective on Y-chromosome diversity. *Proc. Nat. Acad. Sci.* **98**, 10244–10249 (2001).
- Hammer, M. F. *et al.* Dual origins of the Japanese: common ground for hunter-gatherer and farmer Y chromosomes. *J. Hum. Genet.* **51**, 47–58 (2006).
- Shi, H. *et al.* Y chromosome evidence of earliest modern human settlement in East Asia and multiple origins of Tibetan and Japanese populations. *BMC Biol.* **6**, 45 (2008).
- Soares, P. *et al.* Climate change and postglacial human dispersals in Southeast Asia. *Mol. Biol. Evol.* **25**, 1209–1218 (2008).
- Peng, M.-S. *et al.* Inland post-glacial dispersal in East Asia revealed by mitochondrial haplogroup M9a'b. *BMC Biol.* **9**, 2 (2011).
- Zhao, M. *et al.* Mitochondrial genome evidence reveals successful Late Paleolithic settlement on the Tibetan Plateau. *Proc. Nat. Acad. Sci.* **106**, 21230–21235 (2009).
- Qin, Z. *et al.* A mitochondrial revelation of early human migrations to the Tibetan Plateau before and after the last glacial maximum. *Am. J. Phys. Anthropol.* **143**, 555–569 (2010).
- Liu, J. *et al.* Deciphering the signature of selective constraints on cancerous mitochondrial genome. *Mol. Biol. Evol.* **29**, 1255–1261 (2012).
- Chandrasekar, A. *et al.* Updating phylogeny of mitochondrial DNA macrohaplogroup m in India: dispersal of modern human in South Asian corridor. *PLoS One* **4**, e7447 (2009).
- Kang, L. *et al.* Northward genetic penetration across the Himalayas viewed from Sherpa people. *Mitochondrial DNA*, doi:10.3109/19401736.2014.895986 (2014).
- Gayden, T. *et al.* The Himalayas as a directional barrier to gene flow. *Am. J. Hum. Genet.* **80**, 884–894 (2007).
- Cordaux, R. *et al.* Independent origins of Indian caste and tribal paternal lineages. *Curr. Biol.* **14**, 231–235 (2004).
- Wang, H.-W. *et al.* Revisiting the role of the Himalayas in peopling Nepal: insights from mitochondrial genomes. *J. Hum. Genet.* **57**, 228–234 (2012).
- Gayden, T. *et al.* The Himalayas: Barrier and conduit for gene flow. *Am. J. Phys. Anthropol.* **151**, 169–182 (2013).
- Oppitz, M. Myths and facts: Reconsidering some data concerning the clan history of the Sherpas. (1974) Available at: <http://www.dspace.cam.ac.uk/handle/1810/227209> (Accessed: 5th August 2015).
- Peng, Y. *et al.* Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. *Mol. Biol. Evol.* **28**, 1075–1081 (2011).
- Xiang, K. *et al.* Identification of a Tibetan-specific mutation in the hypoxic gene EGLN1 and its contribution to high-altitude adaptation. *Mol. Biol. Evol.* **30**, 1889–1898 (2013).
- Shi, H. *et al.* Y-chromosome evidence of southern origin of the East Asian-specific haplogroup O3-M122. *Am. J. Hum. Genet.* **77**, 408–419 (2005).
- Zhong, H. *et al.* Extended Y chromosome investigation suggests postglacial migrations of modern humans into East Asia via the northern route. *Mol. Biol. Evol.* **28**, 717–727 (2011).
- Karafet, T. M. *et al.* New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. *Genome Res.* **18**, 830–838 (2008).
- Butler, J. M. *et al.* A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. *Forensic Sci. Int.* **129**, 10–24 (2002).
- Yao, Y.-G., Kong, Q.-P., Bandelt, H.-J., Kivisild, T. & Zhang, Y.-P. Phylogeographic differentiation of mitochondrial DNA in Han Chinese. *Am. J. Hum. Genet.* **70**, 635–651 (2002).
- Dames, S. *et al.* The development of next-generation sequencing assays for the mitochondrial genome and 108 nuclear genes associated with mitochondrial disorders. *J. Mol. Diagn.* **15**, 526–534 (2013).
- Andrews, R. M. *et al.* Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.* **23**, 147–147 (1999).

32. Van Oven, M. & Kayser, M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mut.* **30**, E386–E394 (2009).
33. Bandelt, H.-J., Forster, P. & Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48 (1999).
34. Forster, P., Harding, R., Torroni, A. & Bandelt, H.-J. Origin and evolution of Native American mtDNA variation: a reappraisal. *Am. J. Hum. Genet.* **59**, 935 (1996).
35. Saillard, J., Forster, P., Lynnerup, N., Bandelt, H.-J. & Nørby, S. mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *Am. J. Hum. Genet.* **67**, 718–726 (2000).
36. Soares, P. *et al.* Correcting for purifying selection: an improved human mitochondrial molecular clock. *Am. J. Hum. Genet.* **84**, 740–759 (2009).
37. Richards, M., Macaulay, V., Torroni, A. & Bandelt, H.-J. In search of geographical patterns in European mitochondrial DNA. *Am. J. Hum. Genet.* **71**, 1168–1174 (2002).
38. Nei, M. & Gojobori, T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol. Biol. Evol.* **3**, 418–426 (1986).
39. Tamura, K. *et al.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739 (2011).
40. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452 (2009).
41. Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* **22**, 1185–1192 (2005).
42. Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
43. Drummond, A. J., Nicholls, G. K., Rodrigo, A. G. & Solomon, W. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics* **161**, 1307–1320 (2002).

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Author Contributions

B.S. and X.Q. conceived the project. S.B., X.Z., C.C., Bi., O., Ba., Ge., Go., S. L., T.W. and X.Q. collected the samples; S.B., X.Z. and S.L. collected the data; Y.P., H.Z. and K.X. provided technical support in the experiments; S.B., X.Z., H.S., X.Q. and B.S. conducted data analysis and wrote the manuscript.

Additional Information

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