
β-Globin Gene Cluster Haplotype Frequencies in Khalkhs and Buryats of Mongolia

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Abstract β-globin gene cluster haplotype frequencies of 169 Khalkhs and 145 Buryats were estimated, and their characteristics were compared with those of Evenkis, Oroqens, Koreans, Japanese, and three Colombian Amerindian groups. The present study suggests that Colombian Amerindians diverged first from Asian populations and then Buryats diverged from other Asian populations.

Khalkhs form the largest group of Mongols in Mongolia, 75% of the population (*New Encyclopaedia Britannica* 1982). They were traditionally nomadic cattle breeders and hunters and moved a few times a year to seek pasture lands. The old Khalkh society was based on kinship traced through the paternal line. Khalkhs speak a Mongolic language, Khalkha.

Buryats are also Mongols who are distributed widely in Siberia, mainly near the region of Lake Baikal in the Buryat Republic, Russia. They speak a Mongolic language. Some Buryats live in Mongolia and in Inner Mongolia of China, as neighbors of Evenkis, who speak a Tungusic language. Buryats are related to Khalkhs in Mongolia, Mongols in Inner Mongolia and Manchuria, and Kalmyks or Oyrats, who together form the principal Mongolian people, through language ties, history, habitat, and economic type (*New Encyclopaedia Britannica* 1982). Buryats were also traditionally nomadic cattle breeders and hunters.

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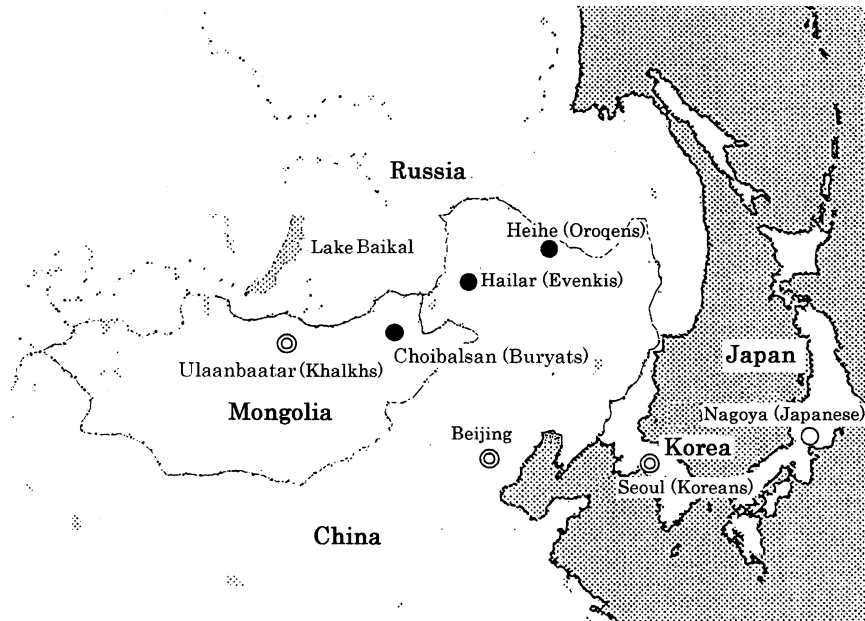


Figure 1. Location of Buryats from Choibalsan and Khalkhs from Ulaanbaatar, where blood samples for DNA were collected.

Their descent is traced through the paternal line, as in Khalkhs. Eastern Buryats have been under the close influence of Khalkhs (*New Encyclopaedia Britannica* 1982).

Genetic relationships between Khalkhs and Buryats have not been studied thoroughly; in particular, there are no data for their β -globin gene cluster haplotypes. In this paper we describe the characteristics of the β -globin gene cluster haplotype frequencies in Khalkhs and Buryats and compared them to those of other Asian populations. We show that haplotype frequency distributions may be more useful than 5' subhaplotype frequency distributions, which have been described most frequently worldwide, in determining genetic relationships among human populations (Shimizu et al. 2004).

Materials and Methods

DNA samples were obtained from nucleated cells from 10 ml of circulating blood of volunteers: 169 Khalkhs who lived in the Ulaanbaatar area of Mongolia and 145 Buryats who lived in the Choibalsan area of Mongolia (Figure 1). The study participants gave their informed consent.

The procedure for the determination of the 5' and 3' subhaplotypes and haplotypes using the PCR method (PCR-RFLPs) was that of Shimizu et al. (2001). Estimations of subhaplotypes and haplotypes were carried out using the EH program of Ott (2003). The heterogeneities of the 5' subhaplotypes and the haplotypes were calculated according to the method of Nei (1987), and the genetic distances (D_m) based on the distribution frequencies of the 5' subhaplotypes and haplotypes were also calculated following Nei (1987).

Results and Discussion

The genotype frequency values of the seven restriction site polymorphisms or RFLPs (restriction fragment length polymorphisms) in the β -globin gene cluster of Khalkhs and Buryats were comparable to the expected values under Hardy-Weinberg equilibrium (chi-square test, $p > 0.05$).

The most frequently encountered 5' subhaplotype in Khalkhs and Buryats was type 2 (+ - - - -) (Table 1). Seventy-five Khalkhs and 42 Buryats were homozygous for the type 2 subhaplotype ($f = 0.67$ and $f = 0.54$, respectively). One Khalkh was homozygous for the type 6 subhaplotype (- + + - +) ($f = 0.08$), and five Khalkhs were homozygous for the type 5 subhaplotype (- + - + +) ($f = 0.17$). Two Buryats were homozygous for the type 6 subhaplotype ($f = 0.12$), and five Buryats were homozygous for the type 5 subhaplotype ($f = 0.19$). One Buryat was homozygous for the type 1 subhaplotype (- - - - -) ($f = 0.08$), and another Buryat was homozygous for the type 12 subhaplotype (+ + - - -) ($f = 0.08$). The type 1 subhaplotype was observed in 11 chromosomes of Buryats, whereas it was found in only one chromosome in Khalkhs. Their chromosomes were present as heterozygous forms for type 1 and 2 subhaplotypes, except one Buryat, who was homozygous for the type 1 subhaplotype. No type 3 chromosomes (- - - - +) were observed in Buryats and Khalkhs, as in the cases of Evenkis, Oroqens, Japanese, and Koreans.

Types 1 and 2 have been thought to be ancestral subhaplotypes (Long et al. 1990; Chen et al. 1990). The type 1 subhaplotype has been observed in some populations at low frequencies (0.004–0.046) (Shimizu et al. 2001). The type 2 subhaplotype is observed most frequently in most human populations, except Bantu and !Kung San Africans, in which the type 3 subhaplotype is most frequently encountered (Ramsay and Jenkins 1987). Interestingly, Vincek et al. (1994) found that chimpanzees and gorilla have only type 3 chromosomes, and they have this type as a homozygous form. Vincek and colleagues concluded that the ancestral human 5' subhaplotype is the type 3 subhaplotype instead of type 1, and they supported the hypothesis that places the present human species in sub-Saharan Africa (Vincek et al. 1994).

The 5' subhaplotype diversity value for Khalkhs was lowest (0.488) among populations examined in the present study, whereas the value for Buryats was highest (0.624). Statistically significant differences in these values were observed

Table 1. Haplotype Frequencies of the β -Globin Gene Cluster of Khalkhs and Buryats Compared to Those of Evenkis, Oroqens, Japanese, Koreans, and Three Colombian Amerindians (Wayuu, Kamsa, and Inga Tribes)

Haplotypes: 5' sub 3' sub	Khalkhs	Buryats	Evenkis ^a	Oroqens ^a	Japanese ^b	Koreans ^b	Wayuu ^b	Kamsa ^b	Inga ^b
1a. - - - - + +	0.001	0.025		0.019					
1b. - - - - + -	0.003	0.004				0.021			
1c. - - - - - +		0.012	0.005		0.005				
2a. + - - - + +	0.150	0.085	0.118	0.094	0.109	0.096	0.406	0.343	0.500
2b. + - - - + -	0.148	0.067	0.237	0.139	0.138	0.189	0.023	0.018	0.033
2c. + - - - - +	0.389	0.413	0.316	0.341	0.308	0.335	0.230	0.319	0.100
3a. - - - + + +							0.034		0.033
3c. - - - + - +								0.019	0.033
4a. - + - - + +		0.003	0.012		0.008		0.016		
4b. - + - - + -		0.007			0.020	0.007			
4c. - + - + + +	0.006	0.010	0.007					0.027	0.033
5a. - + - + + -	0.066	0.084	0.029	0.023	0.061	0.082	0.055		
5b. - + - + + +		0.051	0.011		0.027	0.044	0.008		
5c. - + - + + -	0.090	0.057	0.040	0.119	0.151	0.081	0.013	0.045	
5d. - + - + + -	0.004				0.006		0.005		
6a. - + - + + +	0.020	0.033	0.010		0.025	0.022	0.032	0.023	
6b. - + - + + -	0.045	0.022		0.038	0.037				
6c. - + - + + +	0.062	0.076	0.088	0.140	0.053	0.105	0.086	0.094	0.200
7a. - + - - - +		0.003							
7b. - + - - - -				0.012					
7c. - + - - - +							0.016	0.009	
7d. - + - - - -		0.003							
8b. + - - + + -			0.009						
8c. + - - + + +			0.004						0.009
9a. - + + + + +			0.009						
10a. + + - + + +	0.003			0.012					0.015

between Khalkhs and Buryats, Japanese (0.617), or Oroqens (0.622) and between Buryats and Evenkis (0.533) or Kamsa (0.518) (*t* test, $p < 0.05$).

The lowest values of Nei's genetic distance (2×10^{-3}) based on the 5' subhaplotype frequency distributions were observed between Buryats and Japanese or Koreans, between Kamsa and Wayuu, and between Evenkis and Wayuu or Kamsa. Interestingly, the genetic relationships of Evenkis to two Colombian Amerindian groups were close (Shimizu et al. 2004). The greatest distance (39×10^{-3}) was found between Japanese and Inga, and the next greatest distance (28×10^{-3}) was found between Inga and Buryats or Koreans and between Japanese and Kamsa. The D_m value for Khalkhs in relation to Buryats was 9×10^{-3} , as was also seen between Khalkhs and Oroqens or Kamsa.

The 3' subhaplotype frequencies of the β -globin gene cluster in Khalkhs and Buryats are also shown in Table 1. The 3' subhaplotype d (— —), which has been described in only a few populations (Shimizu and Harihara 2004), was estimated to be present in one chromosome of Khalkhs and in one chromosome of Buryats.

Haplotype 2c (+ — — — — +) was most frequently observed in Khalkhs and Buryats, as shown in Table 1. Haplotype 2b (+ — — — — + —) was not as frequently encountered in Buryats as in other Asian populations. Haplotype 2a (+ — — — — + +) was frequently observed in Colombian Amerindians compared to Asian populations, including Khalkhs and Buryats. Twenty Khalkhs and 12 Buryats were homozygous for haplotype 2c ($f = 0.34$ and $f = 0.29$, respectively). Four Khalkhs and one Buryat were homozygous for haplotype 2b ($f = 0.17$ and $f = 0.08$, respectively), whereas four Khalkhs and one Buryat were homozygous for haplotype 2a. One Khalkh and one Buryat were homozygous for haplotype 5a (— + — + + +). One Buryat and one Khalkh were homozygous for haplotypes 6c (— + + — + — +) and 6a (— + + — + +), respectively. These values from the frequencies of the homozygotes in Khalkhs and Buryats were not so different from the frequency values shown in Table 1, except for haplotype 2c in Buryats.

The haplotype diversities in Colombian Amerindians were lowest (0.693–0.768) among nine populations in the present study, whereas the highest diversity was observed in Japanese (0.841). A statistically significant difference in the haplotype diversity value was found only between Khalkhs (0.786) and Japanese (*t* test, $p < 0.05$).

A low D_m value ($7\text{--}9 \times 10^{-3}$) based on the haplotype frequency distribution was seen between Khalkhs and Buryats, Oroqens, Koreans, or Japanese, as was found between Koreans and Evenkis, Oroqens, or Japanese. However, the value of D_m for Evenkis in relation to Japanese, Oroqens, or Khalkhs was greater than 12×10^{-3} . The genetic relationship between Evenkis and Koreans seems to be close, but the Evenkis are not close to other Asian populations or to Colombian Amerindians. D_m values based on the haplotype frequency distribution also show that the genetic relationships between Khalkhs and Oroqens, Koreans, or Japanese are closer than those between Buryats and these populations. High D_m

values ($36\text{--}154 \times 10^{-3}$) were observed between Colombian Amerindians and Asian populations.

Using classical genetic markers such as blood groups, serum proteins, and red cell enzymes, Novoradovsky et al. (1993) reported genetic similarities between Buryats and Mongols, and they thought that Buryats derived from Mongols. An mtDNA study by Derenko et al. (2000) revealed that Buryats clustered closer to Koreans than to other populations, which was suggested by the present 5' subhaplotype frequency distribution study. Y-chromosome DNA analyses by Santos et al. (1999) and Pakendorf et al. (2002) also found distinct genetic differences between Buryats and Mongols. However, another mtDNA study by Pakendorf et al. (2003) revealed that Buryats had close genetic affinities with Mongols. No genetic studies have been carried out in Buryats from Mongolia, but the genetic differences among many Buryats groups in Russia have been reported to be small (Novoradovsky et al. 1993). Our present study does not show a close genetic relationship between Khalkhs and Buryats, but our haplotype analysis suggests that Colombian Amerindians first diverged from Asian populations and then Buryats diverged from other Asian groups, which has already been suggested by Matsumoto (1988) through *GM* studies.

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