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Short Communication

Analysis of Genetic Differentiation at *ABO* and *Rh* Loci among the Pashtun Populations Inhabiting Lower Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The study of Pashtun populations of North-West Pakistan deserves special attention due to the demographic transitions caused by recurrent displacements, fragmentation and migrations. In order to observe the genetic differentiation in lower Khyber Pakhtunkhwa (KPK) region, ABO and Rh blood groups record of nine Pashtun populations, *i.e.*, Bannu, Dera Ismail Khan, Hangu, Karak, Kohat, Kurram Agency, Lakki Marwat, South Waziristan Agency, and Tank, was collected. Absolute gene diversity, gene differentiation and g*enetic* diversity were estimated to be 0.01, 0.019 and 0.515, respectively. There were the highest affinities between the samples obtained from Lakki Marwat and Tank districts, whereas the least affinities were evident between Karak and South Waziristan Agency populations. Collectively, these results show the presence of sub-populations in lower KPK which may suggest a disturbed nature of these populations. Further studies are needed to understand the nature of differentiation between these Pashtun populations.

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Authors' Contributions
SM conceived and designed the study. AUR and SA collected the data. AUR and SM analyzed the data. SM drafted the manuscript.

Key words
Gene diversity, ABO, Rh, Blood groups, Pashtuns.

The Pashtun is the predominant and a highly diverse population in Khyber Pakhtunkhwa (KPK) province of Pakistan. The study of their genetic structure deserves special attention due to demographic transition caused by displacements, fragmentation and migrations (Rehman *et al*., 2015; Ahmad *et al*., 2016). There are, however, very few data available on the bio-demographic and genetic aspects of Pashtun populations. Previously, Ali and Malik (2015) used *ABO* and *Rh* blood polymorphisms in order to evaluate the differentiation of populations of upper KPK. In this communication, we present our observations on blood group polymorphisms in populations of lower KPK.

Materials and methods

The blood groups record of nine populations/Districts comprising 15,241 individuals was collected (Table I). The phenotypic data of six populations, *i.e.*, Dera Ismail Khan (DI Khan), Hangu, Kurram Agency, Lakki Marwat, South Waziristan Agency (SWA), and Tank, were taken from the respective District Headquarters Hospitals. The information on three populations, *i.e.*, Bannu, Karak, and Kohat, was available in the literature (Hina, 2007; Guloon, 1997).

The maximum likelihood estimate of allele frequencies at the *ABO* locus was obtained through Bernstein method and the concordance with Hardy-Weinberg equilibrium (HWE) was tested by Chi-test (Strickberger, 2005; Falconer and Mackay, 1996). For the appreciation of gene diversity, Nei's concept of degree of differentiation (G_{ST}) and absolute gene diversity (D_{ST}) was employed (Nei, 1978). A genetic distance (DA) matrix was generated through the allele frequencies (Nei and Roychoudhury, 1982). The results were displayed through Principal Component Analyses (Ali and Malik, 2015; Alsuhaibani *et al*., 2015).

Results

The maximum likelihood estimate of allele frequencies at the *ABO* locus showed that *A[p]* allele was the lowest in DI Khan (0.16) and the highest in SWA (0.297), *B[q]* allele was the lowest in Kurram Agency (0.168) and the highest in Karak (0.291), and *O[r]* allele was the lowest in SWA (0.416) and the highest in DI Khan population (0.599). At the *Rh* locus, *d* allele had the lowest estimate in SWA (0.175) and the highest in Karak (0.489) (Table I). Allelic frequencies at the *ABO* locus in five populations and in the total sample were not in agreement with the Hardy-Weinberg equilibrium. The coefficient of variance (CoV) was the highest for *A[p]* allele, followed by *B[q]* and *O[r]* alleles (0.191, 0.152 and 0.11, respectively). The total heterozygosity at the studied loci was observed to be minimum in DI Khan (0.46) and maximum in Karak (0.57).

The allelic systems studied in the populations of lower KPK were compared with that of upper KPK

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Table I.- Allelic frequency and heterozygosity estimates at *ABO* and *Rh* loci in lower KPK populations.

Population	Sample size (n)	ABO Locus			Rh locus Rh-(d)	Average Hetero zygosity
		p[A]	*q[B]*	*r[O]*		
Bannu*	2,581	0.220	0.255	0.525	0.328	0.53
DI Khan*	5,228	0.160	0.241	0.599	0.241	0.46
Hangu	1,853	0.207	0.228	0.565	0.288	0.50
Karak	310	0.239	0.291	0.470	0.489	0.57
Kohat*	1,079	0.233	0.263	0.504	0.282	0.51
Kurram Agency*	1,961	0.248	0.168	0.584	0.242	0.47
Lakki Marwat	660	0.178	0.261	0.561	0.330	0.51
SWA*	651	0.297	0.287	0.416	0.175	0.47
Tank	918	0.186	0.285	0.529	0.325	0.52
Total*	15,241	0.201	0.240	0.559	0.281	0.50

*Significant deviation from Hardy-Weinberg expectations.

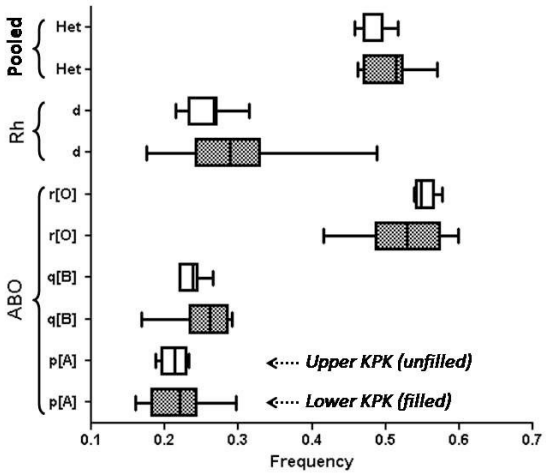

Fig. 1. Box-and-whisker plots showing the range of allelic frequencies and heterozygosities (Het) between populations of lower KPK (filled boxes) and upper KPK (unfilled boxes).

populations (Ali and Malik, 2015). Generally, the allele frequencies depicted wide ranges in populations of the lower KPK when compared to the upper KPK (Fig. 1). For instance, at the *ABO* locus, the upper bounds of *A[p]* allele were quite higher in the lower KPK populations compared to the upper KPK (0.297 *vs.* 0.232); and *B[q]* allele demonstrated a very wide range in comparison to the upper KPK (0.168-0.291 vs. 0.22-0.265, respectively; p=0.291). At the *Rh* locus, *d* allele exhibited wide variability in the lower KPK compared to the populations of the upper KPK (0.175-0.489 vs. 0.215-0.314; unpaired t-test: p=0.285).

Table II.- Gene diversity analysis of lower KPK populations.

Population	Locus	H_T	H_S	D_{ST}	G_{ST}
Group-1 (Bannu, Lakki Marwat, Tank, DI Khan, SWA)	ABO	0.609	0.603	0.006	0.011
	Rh	0.403	0.395	0.008	0.019
	Pooled	0.506	0.499	0.007	0.014
Group-2 (Kohat, Karak, Hangu, Kurram Agency)	ABO	0.608	0.604	0.004	0.007
	Rh	0.439	0.420	0.019	0.042
	Pooled	0.524	0.512	0.011	0.022
All nine populations	ABO	0.609	0.603	0.006	0.010
	Rh	0.420	0.406	0.014	0.032
	Pooled	0.515	0.505	0.010	0.019

Gene diversity at *ABO* and *Rh* loci was evaluated and for this purpose, nine populations were lumped into two groups on the basis of their geographic neighborhoods: Group-1 comprised Bannu, DI Khan, Lakki Marwat, SWA, and Tank populations; while Group-2 consisted of Hangu, Karak, Kohat and Kurram Agency. The total genetic diversity (H_T) was higher in Group-2 compared to Group-1 (0.524 vs. 0.506) (Table II). The absolute gene diversity (D_{ST}) was also higher in Group-2 than Group-1 (0.011 vs. 0.007). Correspondingly, the coefficient of inter-population gene differentiation (G_{ST}) was higher in Group-2. Hence, these analyses showed that the differentiation was much higher in Group-2 populations compared to Group-1. Among the total samples, absolute gene diversity was 0.010 and gene differentiation 0.019. Nei's genetic distance (DA) estimation showed highest affinities between samples from Lakki Marwat and Tank districts (DA=0.0003), between Bannu and Tank (0.0006),

and between Bannu and Kohat districts (0.0007) (Fig. 2). On the other hand, there were the least affinities between the samples from Karak and SWA (0.0303), and between Karak and Kurram Agency (0.0226).

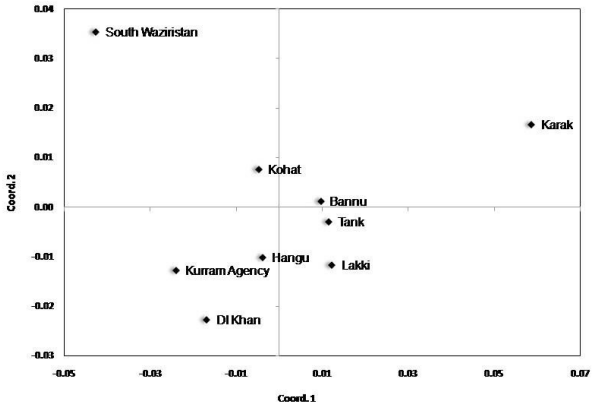

Fig. 2. Scatter plot depicting the output of Principal Component Analysis based on genetic distance matrix in the Pashtun populations.

Discussion

In this study, populations of lower KPK were put into two reasonable groups in order to appreciate the hierarchical decomposition of gene differentiation. Generally, G_{ST} is higher at higher hierarchies, *i.e.*, main regions are genetically more differentiated than the smaller regions within them (Beckman *et al*., 1972). It was hypothesized that total G_{ST} would be higher than the G_{ST} estimates for Group-1 and Group-2. However, the analyses revealed that the G_{ST} in Group-2 was higher than that for total populations of lower KPK (0.019 *vs.* 0.022, respectively), which contradicts the hypothesis described above. This may mean that either the combination of populations in groups does not reflect the actual intra-ethnic genetic structure of the subdivided populations or complex micro-evolutionary events in the subdivided population in a limited geographic area were operational. It was further witnessed that G_{ST} was much conspicuous in lower KPK populations compared to the reported estimates in upper KPK (0.019 *vs.* 0.002, respectively) (Ali and Malik, 2015).

Conclusion

There was a higher level of differentiation in the population of lower KPK at the studied loci. The population appears to be split into various sub-populations which may have their own breeding structures. It may be an indicator of demographic transitions in this region. This is an interesting finding which needs to be scrutinized with the help of highly polymorphic microsatellite markers in an extended sample size representing all fractions of Pashtun ethnicities.

Statement of conflict of interest

Authors have declared no conflict of interest.

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