

Research Report

Genetic affinity of Muslim population in South India based on HLA-DQB1 and relationship with other Indian Populations

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Abstract - The present study made an attempt to observe genetic affinity of the Muslim population in South India with other neighbor populations. In this regard, DQB1 loci of HLA class II gene as a common genetic marker in phylogenetic assessment has been examined in 45 unrelated healthy individuals using sequence-based typing. The result of this study indicates a close genetic similarity among Indian sub-populations, in spite of segregation with other Muslim populations in North India. Although results of present study indicates genetic relationship of selected populations, all HLA loci or at least all loci of each classes to be assessed in order to attain highly probability of estimates.

Keywords: South Indian Muslims, Anthropology, HLA

Introduction

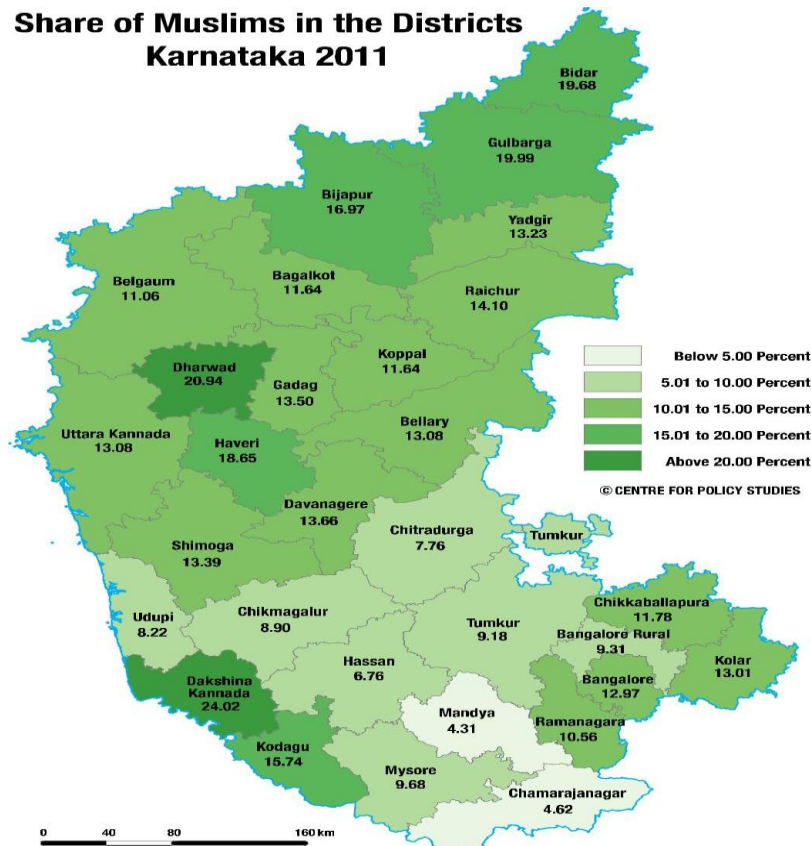
Human leukocyte antigen (HLA) genes are highly polymorphic genes in human which are considered widely as a useful autosomal genetic marker beside the traditional sex chromosomes markers like as mtDNA and Y chromosome for population relationship and phylogenetics. HLA genes are regarded as a useful marker for immunologist in the clinical context and anthropological purpose to investigate the genetic structure of human population and determine affinities between neighboring populations (Sanchez-Mazas 2001; Spínola et al. 2011). HLA genes widely analyzed as useful markers to identify genetic relationship among populations by molecular anthropologist. Genetic relationship and similarity of HLA class II gene between Muslim population in South India in Karnataka state and neighbor populations has been aimed for the present study.

Material and Methods

Population Background & Cultural Characteristics

Population under the study inhabited in Srirangapatna (also spelled Shrirangapattana) located near the city of Mysore in Karnataka state at the Southern region of India. Srirangapatna is an important historical area with considerable religious, spiritual, natural, and cultural background. After Hinduism, Islam is second largest religion in India. According to 2011 census, 172 million of country's population constitutes from Muslims (14.2%). Accordingly, India has the third largest population of Muslims in the world, after Indonesia and Pakistan. The arrival of Muslims in South Asia traced back to Arab traders entry to Malabar (geographic area in Southwest India, covering the Kerala), during the early 7th century, even very earlier than Islam inter to India. Although entry of the Arab Muslim into India around thousand years ago take placed from North-western India and expand to the south and east. The pattern of Muslims entering to India can be considered in three successive waves, the Arabs in 8th century, multiple Turkish people arrived around 1000 C.E., and the Mughal dynasty that entered in 1526. This cycle continued till intrusion of the British who would present India with a new cultural challenge. Share of Muslim

populations in Karnataka increased from 9.87 in 1961 to 12.92 percent (2011) in the population of Karnataka. The share of Muslims in Karnataka has been rising consistently between 1961 and 2011, except a slight decline in the first decade following Independence. Current distribution of Muslims across the State has been shown in Map.1.



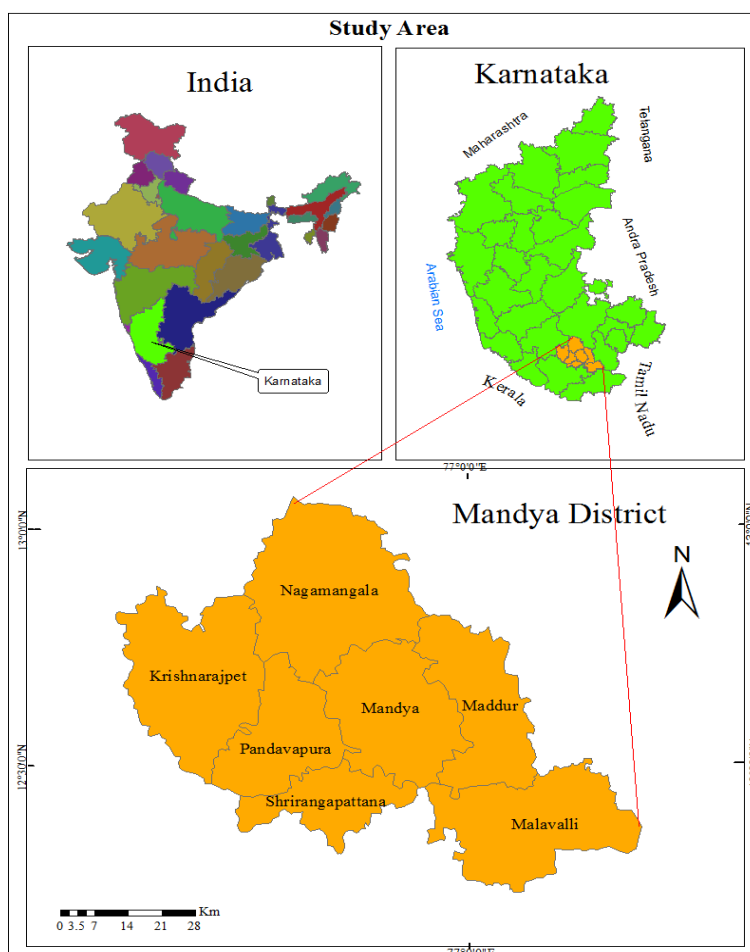
Map.1 Muslims populations distribution in Karnataka State (www.cpsindia.org).

Shrirangapattana Muslims are an endogamous population with Interbreeding and intergroup marriage; cousin or cross-cousin marriages are prevalent among Muslims of Shrirangapattana to keep wealth in their family and to maintain the purity of lineage. For this reasons, population might be carrying specified gene pool that culturally influenced than

neighbor populations. Urdu is the main spoken language of Muslims in Karnataka with Dakhni accent, however most Muslims in Karnataka also speak Kannada, Telugu and Sindhi.

Geography

Srirangapatna town is 12 km² where is located at 12.41° N 76.7° E on the southeast of Mandya district with an average elevation of 679 meters. The area is bounded by Mysore district on its south and southwest, Mandya and Maddur town on its east and Krishnarajanagar on its west. It is situated near the River Cauvery to form an oval shaped island (Map.2).



Map. 2 Field area, Srirangapatna (www.cpsindia.org).

Samples

According to census of 2011, 49% of the population is male and 51% is female. Religion proportion assigned by 74% Hindus and 24% Muslims of the whole population, rest of the population are Christians and Sikhs. Total population size of Muslims is 3102 persons, 1463 males and 1639 females. A total of 45 blood samples from unrelated healthy individuals were taken randomly after obtaining informed consent based on institute ethical committee approval. The origin of all persons was belonging to Muslim family and has been inhabited in this area for at least three generations.

DNA Extraction and Genotyping

Genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and has been checked for the quality by 1% agarose gel electrophoresis. Genomic DNA was amplified at the exon-2 of HLA-DQB1 with the PCR condition of 40 ml reaction mixture subjected to 35 cycles of 95 °C for 3 min, 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. The nucleotide sequences of the PCR products were identified by direct sequencing with both the forward and reverse directions using 3730 DNA analyzer (Big Dye, Applied Biosystems).

Statistical Analysis

All analyses in present study based on allele frequency variation in HLA-DQB1 gene of eleven ethnic groups from India and twenty two populations from all over the different geographical regions that were obtained from (<http://allelefreqencies.net/>) database (González-Galarza et al. 2014). All nucleotide changes were identified by using SeqScape software V2.5 (Applied Biosystems) to detect synonyms and non-synonymous substitution in comparison with the most homologous allele, and allele groups were detected according to IPD-IMGT/HLA Database (<https://www.ebi.ac.uk/ipd/imgt/hla/>) and nomenclature information (Robinson et al. 2014; Marsh et al. 2010). Allele frequencies were obtained via direct counting. DQB1 locus showed a huge variation and many alleles contained very low frequencies. In order to perform a comparison of allele frequencies among various populations, it was necessary in some cases, to consider allele groups in the analysis, losing in part the high power of resolution of this study (Piancatelli et al. 2004). To overcome this problem, all the alleles were classified in 5 corresponding two-digit lower resolution allele groups of HLA-DQB1 to use in Arlequin vr3.5

(Excoffier and Lischer 2010) and SPSS, v 17.0 (SPSS, Inc.). Analysis of molecular variance (AMOVA) to estimate the distribution of genetic diversity between and among populations was performed using Arlequin. Neighbour joining trees constructed according to F_{st} distance (Latter 1972) that were computed by Poptree2 (Takezaki et al. 2009) software. Principal component analysis is used for representation of genetic differentiation via multidimensional scaling (MDS) plot (Borg and Groenen 2003) in SPSS. It should be noted that the sample size of present study and some of those in the literature are probably small when compared to the level of variations at studied loci and it is a frequently encountered problem when studying small populations and might impress lower accuracy of estimates and decrease the power of some statistical tests, however it should not substantially affect the robustness of our inferences (Spínola et al. 2011).

Results and discussions

All the literature data and their allele frequency with two-digit lower resolution have been shown in Table 1 & 2. It is to be noted that allele group of *03 is highly frequent among target population followed by DQB1*06, -*05 and -*02 alleles with 0.2440, 0.1780 and 0.1330 values respectively, and subsequently absence of allele group *04 in present study and is very rare among other Indian populations (Agrawal et al. 2008; Kohaar et al. 2009; Seshasubramanian et al. 2018) with lowest frequency as well (Table1). Rarity of DQB1*04 allele is visible in other South and West Asian populations (Malavige et al. 2007; Mohyuddin et al. 2002; Farjadian et al. 2004) and also in some Southeast Asian (Hoa et al. 2008; Sanchez-Mazas 2006; Zhao et al. 1993) countries than other world populations. One interpretation for discrepancy in allele combinations of HLA genes between different populations is the complementary and compensatory abilities of allelic products encoded by the haplotype to bind peptide epitopes from different pathogens, another being past population differentiations or recent admixture of populations (Sanchez-Mazas et al. 2011).

Although, there are some differences among Indian subpopulations, the results of molecular variance analysis (AMOVA) strongly confirmed that genetic diversity among Indian ethnic groups was mainly confined to intra-subpopulation variations and revealed that more than 99% of the variation components were contributed by the within population level and differentiation among populations was extremely low, in addition F_{st} was 0.0063 which indicates little subdivision among the Indian subpopulation, and respectively more than 93% with 0.066

F_{st} for other world populations as well (Table 3 and 4). Genetic relationship among population of present study with both Indian ethnic groups and other countries based on F_{st} genetic distances has been illustrated in Figure 1 and 2 via Neighbor-joining tree. The population under the study has been located in the same cluster in adjacent to New Delhi (ND) population (Kohaar et al. 2009) North Gujarat (NG) population (UPD)¹ and a little far from two Muslim populations of India i.e. Northeast Shia (IS) and Sunni (ISU) populations (Agrawal et al. 2008) that it has depicted in Figure 1, and accordingly, considerable separation in MDS plot results represented no evidence on strong genetic affinity between Muslim groups in Northeast (Agrawal et al. 2008) area and people of object (Fig. 3). As shown in Figure 2, the Muslim population in present study is close to Eastern Asian (Sanchez-Mazas 2006; Zhao et al. 1993) and quite separated from Western Asia. The genetic relationship of target population with different world populations, reflects proximity to Greece (Kokaraki et al. 2009), Singapore (Sanchez-Mazas 2007) and Thailand (Chandanayingyong 2002) populations followed by China (Zhao et al. 1993) and Mongolia (Machulla et al. 2003) and well separated from Northeast of Asia such as Russia (Krylov et al. 1995) and South American countries (Cerna et al. 1993) which is summarizes by MDS plot (Fig. 4) and has been verified by Neighbor-joining tree which has depicted genetic relationship among present study population and other ethnic groups (Fig. 2). To emphasis on association between Indian and non-Indian Muslim populations, genetic distances were calculated between Muslim populations from Iran (Farjadian et al. 2004), Pakistan (Mohyuddin et al. 2002), Jordan (Sánchez-Velasco et al. 2001) and Saudi (UPD) with 0.105, 0.115, 0.039 and 0.054 distance values respectively, from Muslim neighbor countries; and also with Northeastern Indian Muslims (Agrawal et al. 2008), non-Muslim New Delhi population (Kohaar et al. 2009) and North Gujarat (UPD) populations with distance values 0.036, 0.040 (Northeast Indian Muslims), 0.015 and 0.017 respectively (Table 5). Neighbor-joining tree based on Nei's genetic distances demonstrated the distance results (Fig. 5). The results reflect more affinity of our target population to Indian populations especially non-Muslim Indians (with quite low distances), than Muslims in India and other countries that could be estimated their possible admixture with other Indian populations that occurred at different time intervals. These results strengthen the finding of several similar reports according to classical genetic marker such as mitochondrial DNA (Eaaswarkhanth et al. 2010; Khan et al. 2004) that resulted formerly.

¹Data defined by “unpublished data (UPD)” in this study, has been collected from <http://www.allelefrequencies.net>

Table 1 Low-resolution HLA-DQB1 allele frequencies in Indian populations

Populations With sample size (n) & Reference	Alleles with frequency				
	DQB1*02	DQB1*03	DQB1*04	DQB1*05	DQB1*06
NG* (n=338) (<i>UPD</i>)	0.2273	0.3788	0.0152	0.1364	0.2423
ND (n=102) (<i>Kohaar et al., 2009</i>)	0.1860	0.3630	0.0200	0.1810	0.2500
MP (n=45) (<i>present study</i>)	0.1330	0.4440	0.0000	0.1780	0.2440
IB (n=59) (<i>Begovich et al., 2001</i>)	0.1950	0.2160	0.0170	0.2130	0.3590
IA (n=186) (<i>Seshasubramanian et al., 2018</i>)	0.1344	0.3119	0.0376	0.1881	0.3280
IL (n=123) (<i>Gjertson and Terasaki, 1998</i>)	0.2330	0.2710	0.0080	0.2210	0.2670
IK (n=190) (<i>Agrawal et al., 2008</i>)	0.2390	0.2840	0.0210	0.1860	0.2880
IR (n=196) "	0.2650	0.2380	0.0080	0.2250	0.2640
IS (n=190) "	0.2130	0.3110	0.0450	0.2290	0.2020
ISU (n=188) "	0.1970	0.2700	0.0370	0.2890	0.2070
IU (n=202) "	0.2150	0.2700	0.0190	0.2030	0.2930
NR (n=98) "	0.1630	0.2690	0.0200	0.3320	0.2160

*NG: North Gujarat, ND: New Delhi pop 2, MP: Present study, IB: India Bombay, IA: Andhra Pradesh Telugu, IL: India Lucknow, IK: Northeast Kayastha, IR: Northeast Rastogi, IS: Northeast Shia & ISU: Northeast Sunni, IU: Uttar Pradesh. NR: North Rajbanshi.

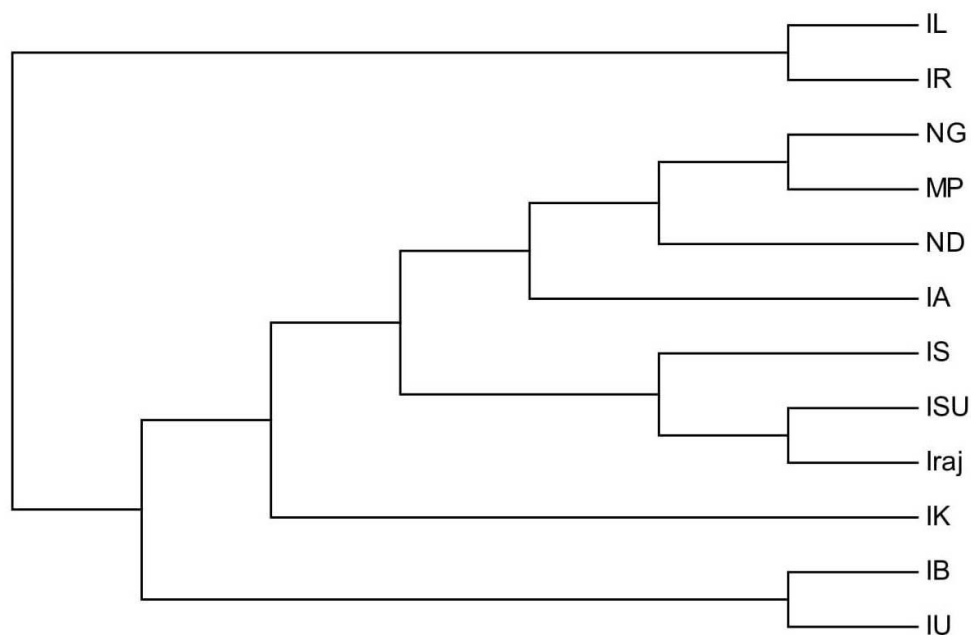


Fig. 1 Neighbor-joining tree showing the genetic relationship among population of present study and other Indian sub-populations.

Table 2 Low-resolution HLA-DQB1 allele frequencies in 35 different world populations

Populations With sample size (n) & Reference	Alleles with frequency				
	DQB1*02	DQB1*03	DQB1*04	DQB1*05	DQB1*06
Vietnam* (n=170) (<i>Hoa et al. 2008</i>)	0.0910	0.5490	0.0410	0.2180	0.1010
Srilanka (n=101) (<i>Malavige et al. 2007</i>)	0.1920	0.1920	0.0100	0.2980	0.3080
Japan (n=371) (<i>Saito et al. 2000</i>)	0.0030	0.3570	0.1540	0.1370	0.3490
Korea (n=324) (<i>UPD</i>)	0.0850	0.3370	0.1160	0.1710	0.2910
MongoliaK (n=200) (<i>Machulla et al. 2003</i>)	0.1750	0.4300	0.0950	0.1200	0.1800
MP (n=45) (<i>present study</i>)	0.1330	0.4440	0.0000	0.1780	0.2440
RussiaSE (n=70) (<i>Krylov et al. 1995</i>)	0.0590	0.7430	0.1000	0.0800	0.0180
BrazilPX (n=74) (<i>Cerna et al. 1993</i>)	0.0000	0.7350	0.2650	0.0000	0.0000
ArgentinaCH (n=54) (<i>UPD</i>)	0.0180	0.7590	0.1500	0.0460	0.0270
SingapoRM (n=132) (<i>Sanchez-Mazas 2007</i>)	0.1090	0.4280	0.0180	0.2630	0.1820
ItalyR (n=100) (<i>Testi and Mariani 1998</i>)	0.1950	0.4250	0.0400	0.1750	0.1650
Greece5 (n=500) (<i>Kokaraki et al. 2009</i>)	0.1270	0.4170	0.0040	0.2770	0.1750
IsraelLj (n=119) (<i>Amar et al. 1999</i>)	0.3330	0.4370	0.0260	0.0660	0.1380
IranYZ (n=65) (<i>UPD</i>)	0.2390	0.3690	0.0620	0.1150	0.2150
Thailand (n=142) (<i>Chandanayingyong 2002</i>)	0.1270	0.3950	0.0460	0.2990	0.1330
IraqE (n=79) (<i>UPD</i>)	0.2152	0.4810	0.0190	0.1329	0.1519
Malay (n=62) (<i>Sanchez-Mazas 2007</i>)	0.0580	0.3840	0.0120	0.3740	0.1720
Saudi (n=499) (<i>UPD</i>)	0.3615	0.2508	0.0188	0.1233	0.2456
PakisB (n=66) (<i>Mohyuddin et al. 2002</i>)	0.3970	0.1120	0.0090	0.3400	0.1420
IranB (n=100) (<i>Farjadian et al. 2004</i>)	0.3200	0.1450	0.0300	0.3800	0.1250
Jordan (n=146) (<i>Sánchez-Velasco et al. 2001</i>)	0.3600	0.2810	0.0000	0.1410	0.2180
Leb (n=191) (<i>Samaha et al. 2003</i>)	0.1950	0.5070	0.0260	0.1990	0.0730
China3 (n=58) (<i>Zhao et al. 1993</i>)	0.0950	0.4990	0.0690	0.0950	0.2420

*Vietnam: Vietnam Hanoi Kinh pop 2, Srilanka: Sri Lanka Colombo Sinhalese, Japan: Japan Central, Korea: South Korea pop 1, Mongolia_K: Mongolia Khalkha, MP: Present study, RussiaSE: Russia Siberia Eskimo, BrazilPX: Brazil Central Plateau Xavante, ArgentinaCH: Argentina Chiriguano, SingaporeRM: Singapore Riau Malay, ItalyR: Italy Rome, Greece5: Greece pop 5, IsraelLj: Israel Libyan Jews, IranYZ: Iran Yazd Zoroastrian, IraqE: Iraq Erbil, Malay: Malaysia pop 2, Saudi: Saudi Arabia pop 4, PakisB: Pakistan Baloch, IranB: Iran Baloch, Jordan: Jordan Amman, Leb: Lebanon pop 2, China3: China Shanghai pop3.

Table 3. Summary of AMOVA analysis based on DQB1 allele frequency of Indian populations

Source of variation	df	SS	VC	PV
Among populations	11	8.277	0.00241	0.64
Within populations	1907	715.599	0.37525	99.36
Total	1918	723.875	0.37766	
Fixation index		FST: 0.00638		

df: degree of freedom, SS: sum of squares, VC: variance components, PV: percentage of variation

Table 4 Summary of AMOVA analysis based on DQB1 allele frequency of different world populations

Source of variation	df	SS	VC	PV
Among populations	22	92.154	0.02485	6.65
Within populations	3646	1271.161	0.34865	93.35
Total	3668	1363.315	0.37350	
Fixation index		FST: 0.06654		

Table 5 Genetic distances of nine pairs of Indian and Indian Muslims and four neighbor country Muslim populations.

	2	3	4	5	6	7	8	9
1. Saudi	0.049	0.054	0.011	0.054	0.015	0.022	0.023	0.032
2. PakisB	****	0.007	0.050	0.115	0.081	0.077	0.054	0.043
3. IranB		****	0.062	0.105	0.073	0.063	0.036	0.025
4. Jordan			****	0.039	0.019	0.029	0.038	0.044
5. Mp*				****	0.017	0.015	0.036	0.040
6. NG					****	0.003	0.013	0.022
7. ND						****	0.007	0.012
8. IS							****	0.003
9. ISU								****

*present study population

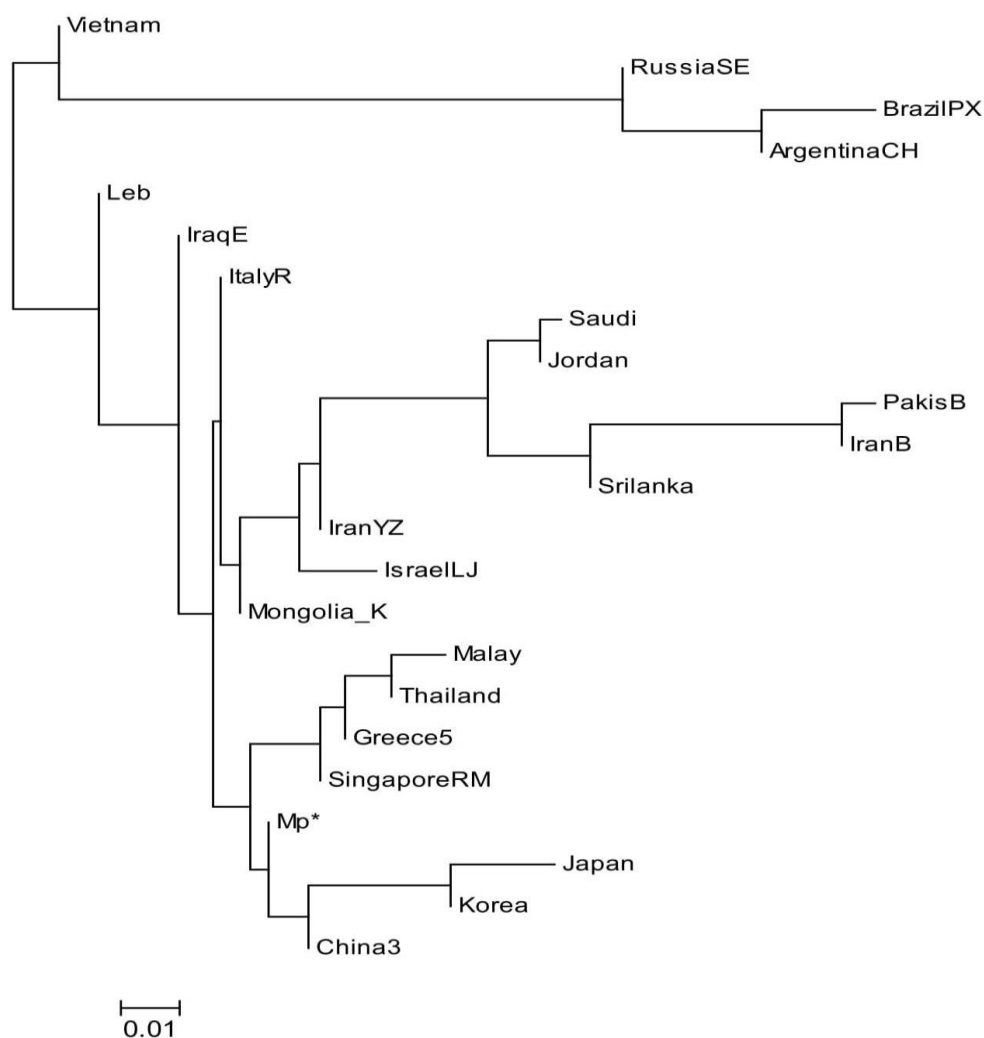


Fig. 2 Neighbor-joining tree showing the genetic relationship among population under study and other world populations.

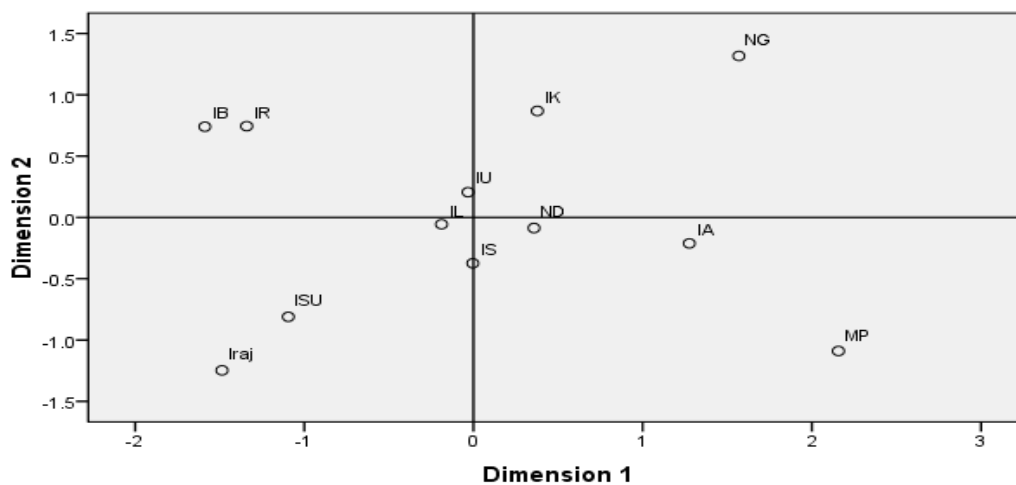


Fig. 3 MDS plot to representation of genetic differences of Indian populations

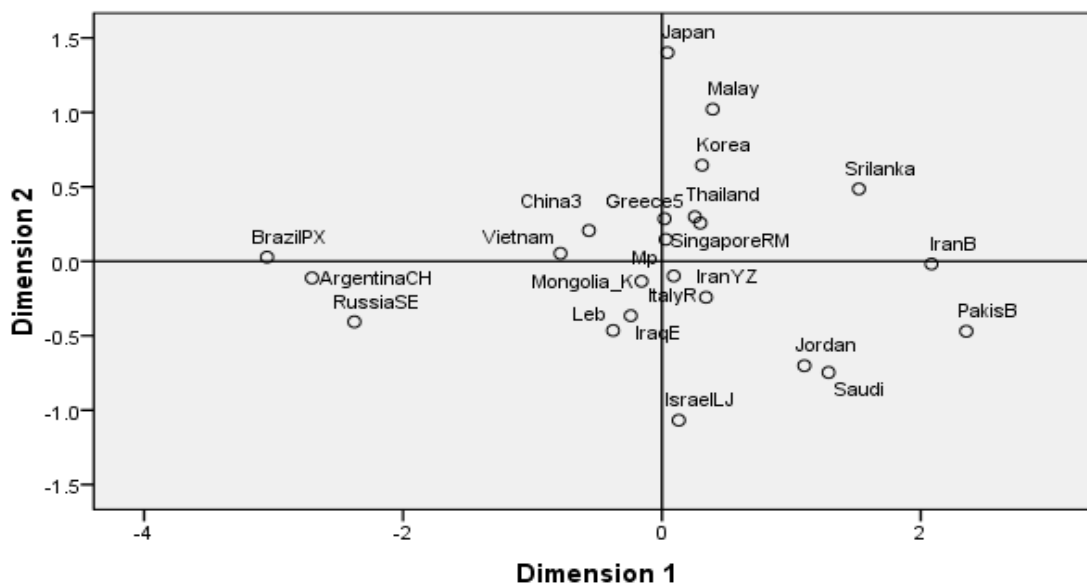


Fig. 4 MDS plot to representation of genetic differences of different world populations

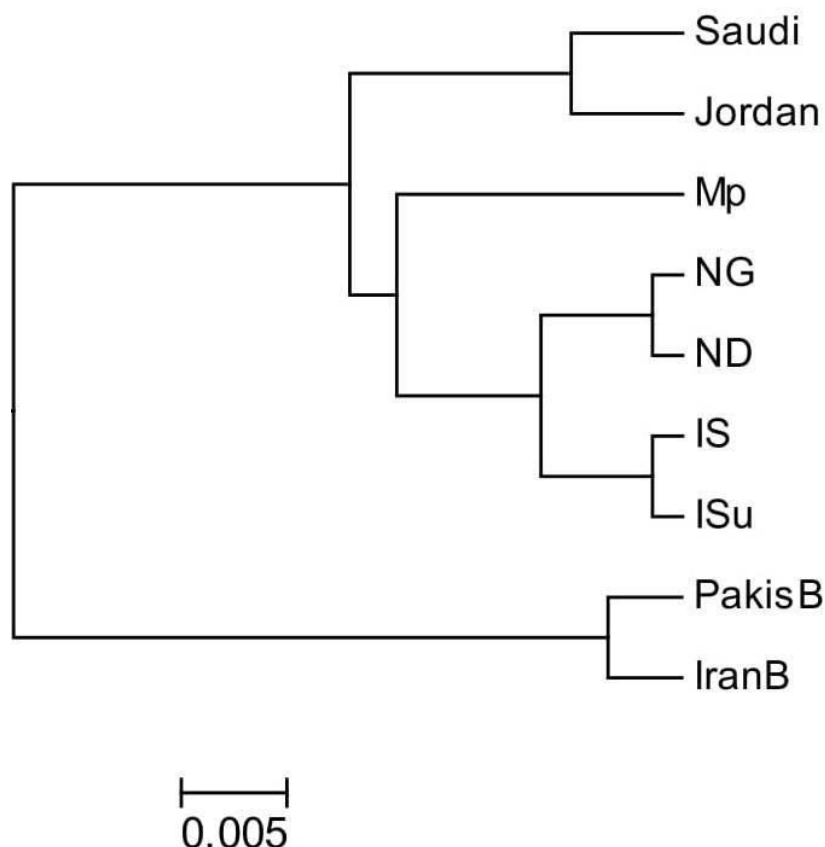


Fig. 5 Neighbor-joining tree showing the genetic relationship among population under the study and Muslims in neighbor country and two other Indian populations based on Nei's genetic distances.

Conclusion

The results of this study demonstrate close relation between Muslims in South Karnataka and other Indian sub-populations although well separation with other Muslim population in North India has been illustrated unlike primary assumption. It can be concluded that Muslim population of present study most probably have been admixed genetically with neighbor populations in Southern region of India. Preferably, the results of present study might be fully explored and deepened when all HLA loci or at least all loci of each classes to be analyzed with high resolution genotyping.

Conflicts of Interest: The authors declare no conflict of interest.

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