Journal of Genetic Engineering and Biotechnology (2016) **14**, 241–245

HOSTED BY

ELSEVIER

Academy of Scientific Research & Technology and National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ORIGINAL ARTICLE

Environmental selection influences the diversity of TLR genes in ethnic Rajbanshi population of North Bengal Region of India

Avishek Das [1], **Pokhraj Guha** [1], **Tapas Kumar Chaudhuri** [*,1]

Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohaunpur, Siliguri, West Bengal 734013, India

Received 28 May 2016; revised 6 September 2016; accepted 20 September 2016
Available online 10 October 2016

KEYWORDS

Toll-like receptors;
Genetic distance;
PCAplot;
Dendrogram

Abstract *Background:* Toll-like receptors are the mediators of the innate immune response to pathogens. In human, this gene family regulates the inflammatory pathways and is associated with the susceptibility to infection.

Subjects and methods: The distribution and the diversity patterns of TLR genes in Rajbanshi population ($n = 85$) who are the inhabitants of the Northern part of West Bengal, have been investigated in the present study. PCR-SSP was done for all the ten TLR genes. We have also constructed the phylogenetic tree principal component analysis and genetic distance for all the four populations.

Results: It has been observed that in Rajbanshi population, the frequency of TLR8 (0.894) is higher and the frequency of TLR2 (0.176) is very low. Dendrogram based analysis, as well as the PCA plot, documented the closeness of Rajbanshi and Gurkha population. However, Rabha is distantly related to Rajbanshi population though evidences suggest their emergence from the same East-Asian lineage. Genetic distances between Rajbanshi–Gurkha and Rajbanshi–Muslim are very much smaller than that of Rajbanshi–Rabha populations.

Interpretations & conclusions: Therefore, it may be concluded that Rajbanshi, Gurkha and Muslims are very much mixed populations and have genetic closeness due to exposure to similar environmental conditions. On the other hand, the Rabhas strictly follow the endogamy and are restricted to a particular region and therefore maintain considerable distances with the other three populations. The data showed some interesting observations which deviate the contemporary thought in respect to the population genetics.

1. Introduction

Human populations around the world are not only extremely diverse but also show wide adaptability to their respective local environment. Such kind of adaptation is needed not only due

* Corresponding author.
E-mail addresses: dasavishekdas2012@gmail.com (A. Das), pokhraj_guha05@yahoo.co.in (P. Guha), dr_tkc_nbu@rediffmail.com (T.K. Chaudhuri).
[1] These authors contributed equally to this work.

Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2016.09.006

to difference in their food habits but also due to the development of tolerance against the microbial world around us [12]. Genetic markers play a very important role in the study of the gene-disease and gene-environment interactions. Balancing selection is a major factor which shapes the innate immune system. Furthermore, genes of the immune system are under constant evolutionary pressures [5]. The immune-related genes keep on changing during the evolutionary process because of their continuous encounter with the environmental antigens thereby influencing disease susceptibility [1,15]. This study proves how the environmental pathogens influence the modification or change of different markers under different conditions. TLRs are among those markers which can specifically recognize the conserved molecular patterns like Pathogen Associated Molecular Patterns (PAMPs) and danger-associated molecular patterns (DAMPs) [3]. Genetic variations in TLRs may induce or inhibit the susceptibility of some diseases. It was also proved that strong pressures exerted by infectious diseases like plague may influence the convergent evolution of some of the TLRs in some human populations (European and Rroma) in recent times [13]. The present investigation has been aimed to study the frequency pattern of ten TLR genes in Rajbanshi population in North Bengal Region of India. Rajbanshi population is an ethnic caste group found in North Bengal and neighboring areas [11]. Koch and Rajbanshis are actually two different tribes but united by the great king into one and named as Koch-Rajbanshi [11]. They are the inhabitants of Jalpaiguri and Cochbehar districts of North Bengal. Previous studies on HLA and KIR genes revealed the influence of East Asian lineages on the Rajbanshis [14,2,9]. Their main dialect is Bengali in this region and they are mainly Hindu in their religion. However, recently this population has mixed with the Indo-Aryan and with the Dravidians lineages [4]. Herein, the frequency and distribution of 10 TLR genes in the ethnic Rajbanshi population have been studied to find out how the local environmental pressure/selection shapes the TLRs profile of a population.

2. Materials and methods

2.1. Study design

Blood samples were collected (n = 85) from the region of Cooch Behar, Jalpaiguri and adjacent areas of Siliguri where they inhabit mostly (26° 20′–27° 03′ N and 88° 18′–89° 29′ E). The samples were collected on the basis of their ethnicity, caste and health conditions. Individuals having three generations of common pedigree were excluded from the analyses. All the donors were informed regarding the purpose of the study before the collection of the blood samples and written consents were provided by the volunteers. The investigation was approved by the Human ethics committee of the Department of Zoology, University of North Bengal (Zoo/4133/2011).

Blood sample (3 ml) was taken from each volunteer by vein puncture method under the guidance of a medical practitioner and was stored in EDTA containing vials at −20 °C until use. Genomic DNA was extracted from the samples by the standard Phenol–Chloroform extraction method with slight modifications. This was followed by PCR-SSP typing for all the 10 TLRs (Table 1). Primers were designed based on the conserved regions of the 10 different TLR genes using NCBI BLAST http://www.ncbi.nlm.nih.gov/tools/primer-blast/. Primers were supplied by the Integrated DNA Technologies, Inc, Iowa, USA.

2.2. Statistical analysis

Neighbor-joining tree and Nei's genetic distances have been constructed using POPGENE (ver-1.32) and Phylip (ver-3.5). Chi-square calculation was done using Kyplot (ver-2.0). Principal component analysis (PCA) was done using Sigma plot (ver-13).

3. Results

3.1. Frequency calculation

Observed frequencies of 10 TLR genes in Rajbanshi population as well as in other three populations were calculated. It has been observed that in Rajbanshi population, the frequency of TLR8 (0.894) was highest followed by TLR6 (0.882) and TLR9 (0.882) respectively (Fig. 1 and Table 2). When Rajbanshi population was compared with Gurkha, Muslim and Rabha populations of this region, (Communicated elsewhere) it was found that the frequency of TLR8 was highest among the Rajbanshi population. However, the frequency of TLR4 was highest in Gurkha and Rabha populations. Previously TLR1 to TLR5 genes were screened among the Rabha population [10] where it was found that the frequency of TLR4 was calculated as highest and TLR5 was in least frequency. On the other hand, TLR5 was highest among Muslims (Communicated elswhere). Interestingly, the frequency of TLR2 was very low among all the four populations.

3.2. Chi-square analysis

When Chi-square analysis was performed among the four populations, it was found that there are no significant differences between Rajbanshi and Gurkha when compared with the other two populations. There were no significant differences found for TLR1 and TLR9 when compared between the Rajbanshis and other three populations.

3.3. Genetic distance and PCA analysis

Genetic distance based Neighbor-joining dendrogram was constructed and interestingly, it was found that Rajbanshi, Gurkha and Muslim occupied the same cluster wherein Gurkha and Muslim population were grouped together, while the Rabhas occupied a separate cluster (Fig. 2). It was assessed from the principal component analysis (PCA) that Rajbanshi and Rabha populations are quite distantly placed in the plot whereas Muslim and Gurkha are very close to each other (Fig. 3). Nei's genetic distance was calculated by comparing Rajbanshi with three other populations and it has been found that the distance between Rajbanshi and Rabha was 0.0745, between Rajbanshi and Gurkha was 0.0685 and that between Rajbanshi–Muslim was 0.0694 (Table 3).

Table 1 List of forward and reverse primers for the 10 TLRs in human.

Genes	Forward primers (5′–3′)	Reverse primers (3′–5′)	Product size (bp)	GC content (%)	Tm
TLR1	TCAACCAGGAATTGGAATAC	AGTTCCAGATTTGCTACAGT	382	40	52
TLR2	GGATGGTTGTGCTTTTAAGTACTG	AAGATCCCAACTAGACAAAGACTG	2671	41.67	55.5
TLR3	ATTGGGTCTGGGAACATTTCTCTTC	GTGAGATTTAAACATTCCTCTTCGC	792	44/40	57
TLR4	TTCTTCTAACTTCCTCTCCTGTG	TTAGCTGTTCGGCTCTACTATGG	1087	43/47	58.6
TLR5	CATTGTATGCACTGTCACTC	CCACCACCATGATGAGAGCA	446	45/55	57.6
TLR6	ACAACCCTTTAGGATAGCCACTG	AAACTCACAATAGGATGGCAGG	398	47.83	56.9
TLR7	AGTGTCTAAAGAACCTGG	CTTGGCCTTACAGAAATG	545	44	50.5
TLR8	CAGAATAGCAGGCGTAACACATCA	AATGTCACAGGTGCATTCAAAGGG	637	45.83	58.4
TLR9	TCTAGGGGCTGAATGTGACC	ACAACCCGTCACTGTTGCTT	1106	55	59.8
TLR10	GTCGAAGACCCAATATACAG	ATTAAGCAATAGAACCGATG	954	45/35	52
Growth hormone (positive control)	CTTCCCAACCATTCCCTTA	CGGATTTCTGTTGTGTTTC	424	47/42	50.3

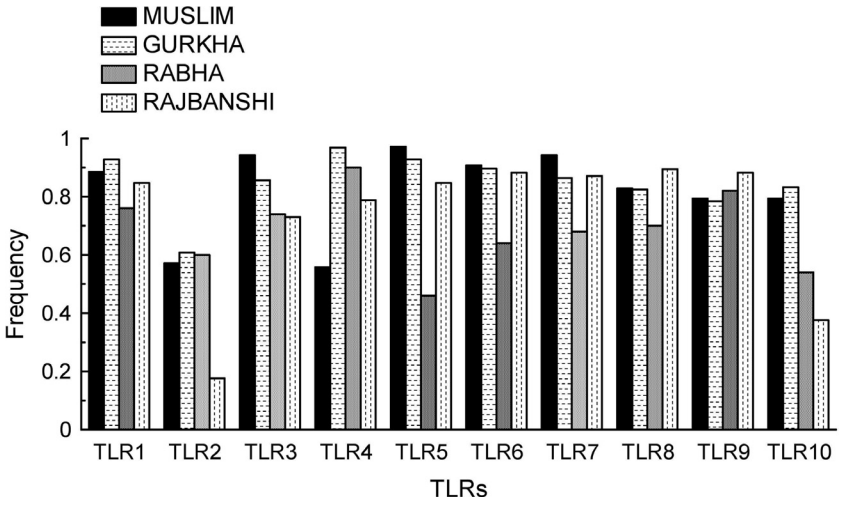

Figure 1 Frequency graph of ten TLR genes was constructed using Kyplot (ver-2.0) of the four populations in the North Bengal region.

Table 2 Observed frequencies of the 10 TLR genes in the four populations. χ^2 values were also mentioned where each gene was compared between two populations for any statistical differences.

	Rajbanshi(RA)	Gurkha(G)	Rabha(R)	Muslim	RAXG	RAXM	RAXR	MXG	GXR	RXM
TLR1	0.847	0.928	0.760	0.886	2.723	0.401	1.056	0.9314	8.020**	3.687
TLR2	0.176	0.608	0.600	0.571	36.63***	32.22***	23.54***	0.229	0.005	0.034
TLR3	0.729	0.856	0.740	0.943	4.385*	18.52***	0.004	4.691*	2.549	13.427***
TLR4	0.788	0.968	0.900	0.557	15.569***	11.33***	2.047	57.426***	2.134	17.499***
TLR5	0.847	0.928	0.460	0.971	2.723	9.99**	20.80***	1.820	45.051***	66.880***
TLR6	0.882	0.896	0.640	0.907	0.007	0.135	9.822**	0.009	14.349***	17.349
TLR7	0.870	0.864	0.680	0.943	0.004	2.700	6.005*	3.927*	6.745**	20.745***
TLR8	0.894	0.824	0.700	0.829	1.458	1.330	6.841**	0.0041	2.591	2.986
TLR9	0.882	0.784	0.820	0.793	2.728	2.364	0.562	0.0006	0.1057	0.0424
TLR10	0.376	0.832	0.540	0.793	44.031***	37.81***	2.789	0.4299	14.6650***	10.612**

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

4. Discussion

Modern Indian populations have originated from two ancestral populations: on one hand "Ancestral North Indians" who are genetically close to Middle Eastern, central Asians and Europeans while on the other hand, "Ancestral South Indians" who have shown proximity to East-Asians lineage [16]. Rajbanshi populations are the indigenous ethnic caste population of Eastern Terai and can also be found in Assam, Bengal and Bihar states of India [11]. Even today, most of the Rajbanshis are found to inhabit in Assam, Meghalaya, Tripura, Nagaland, and Manipur also [18]. Herein we have made an attempt to unveil the TLR profile of this population as it one of the most widely distributed scheduled caste population of Eastern and North Eastern India.

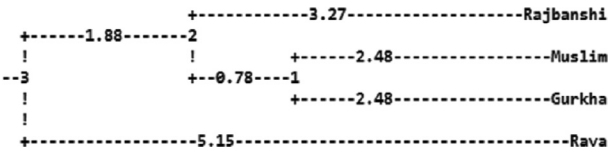

Figure 2 Neighbour joining tree was constructed using POPGENE (ver-1.32) and Phylip (ver-3.5) showing relationship among Rajbanshi and three other populations.

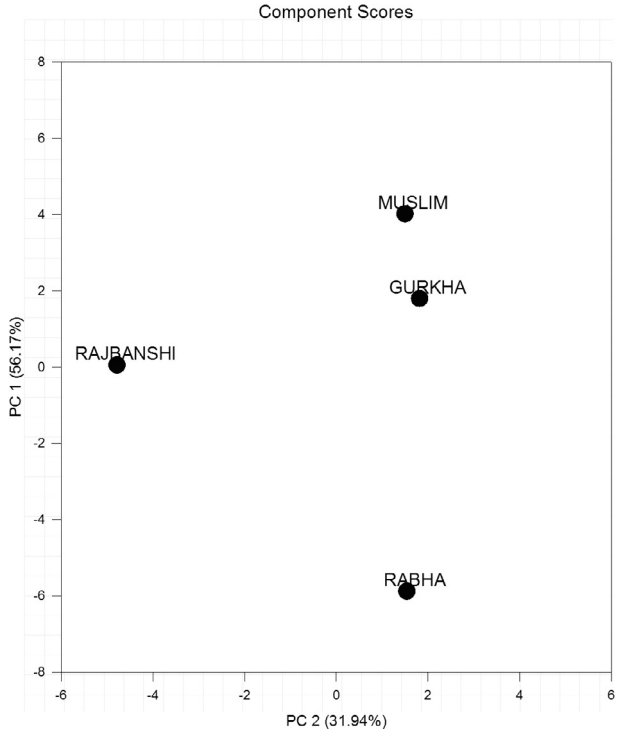

Figure 3 Principal component analyses (PCA) based on observed frequencies of the 10 TLR genes in the four ethnic populations of North Bengal constructed with Sigma plot (ver-13).

Table 3 Nei's genetic distances among the Rajbanshi population using POPGENE (Ver-1.32) software.

POP ID	Rajbanshi	Rabha	Muslim	Gurkha
Rajbanshi	**0.000**			
Rabha	0.0745	**0.000**		
Muslim	0.0694	0.1557	**0.000**	
Gurkha	0.0685	0.0973	0.0526	**0.000**

The bold values $\leq$0.05 signified the similarities of the same populations.

Earlier researches have shown that environmental pathogens have modified the TLR profiles in individuals affected by various diseases like asthma and tuberculosis [19,8]. The selection pressure has also modified the TLR gene pool in various populations in the world [13]. Recent studies have revealed that populations of different genetic ancestry have shown convergent evolution with respect to some of the TLR genes due to the interactions with some infectious diseases like plague in a particular environmental condition [13]. Sometimes polymorphism in the TLRs may also become susceptible or resistant to certain diseases [1,15]. In our previous study, we found that chronic gastritis and associated stomach problems were common in Rabha population and therefore we speculated that there is a strong relationship between KIR and TLR in disease progression [10]. The genetic heterogeneity among the Indian populations have put forward an immense challenge before the researchers of different fields [20]. Subsequently, the strict endogamy practices in the populations of North Bengal along with the evolutionary forces have resulted in higher differences in allele frequencies between the groups, which have remained intact for thousands of years [7,17].

Recently we have screened 10 TLR genes in the ethnic Rajbanshi population of North Bengal and compared with the other three populations of this region. It is interesting to find that the genetic distance between Rajbanshi and Rabha is greater than the Rajbanshi–Gurkha and Rajbanshi–Muslim respectively. But, as per anthropological and genetic records, East Asian lineages have strong influence on the genetic ancestry of Rajbanshi, Rabha and Gurkha respectively [6,9,10] and Muslim population of this region showed their proximity with indigenous non Muslim population along with a small frequency of the Middle East ancestry [20,7]. It was found that the Muslims, the Gurkhas and the Rajbanshis are the residents of the same locality and are therefore exposed to similar environmental effects. On the other hand, the endogamous tribal Rabha population is very much confined in their local environment. Thus the genetic closeness of Rajbanshis with Muslims and distant relationship with Rabha indicated that similar environmental pressure may be responsible for the convergent evolution and selection of the TLR gene pool among the populations inspite of their different genetic ancestry.

The principal component analyses also documented the distant positions of Rabha and Rajbanshi in the score plot while Muslim and Gurkha were close to each other. Researchers all over the world focussed their work on correlating the associations of KIR with various diseases worldwide but concrete study with respect to TLR diversities, distributions and frequencies in different populations of the World are needed on the populations especially in the region of North Bengal where various ethnic endogamous populations are residing. This was also supported by Neighbor-joining dendrogram which suggested the closeness of the Rajbanshis with Gurkhas and Muslims while showing distant relation with the Rabhas. Convergent evolution has occurred in TLR genes among the above-mentioned populations due to the sharing of similar

environmental conditions. It is quite interesting to observe that although the Rajbanshi, Gurkha and Rabha populations have shared ancestry due to their emergence from a common East-Asian stock, there is no similarity in the distribution of TLR genes among them as has been recorded in our present study. However, there exist considerable similarities in the distribution of TLR genes between the Muslim and the Gurkha population who share the same environment but differ considerably in their ethnicity. This striking observation may depict the impact of environmental selection on the distribution of TLR genes. Such influences of the environment on TLR distribution may depend on the constant presence of specific pathogens present in respective environment. Thus, it may be assumed that TLR genes play a significant role in shaping the genetic ancestry of the above mentioned populations from North Bengal region of India as well as in determining disease exposure in these populations.

5. Conclusion

It is also sensible to say that this work is a pioneer work done in the above-mentioned populations on TLRs and diversity study which must be needed around the world on different populations. Such works on TLRs diversity in different populations are very much essential to understand the disease susceptibility and the phenomenon by which local environment creates pressure to change the frequencies and genetic makeup of TLRs.

Authors' contributions

Avishek Das performed the experiments and analyzes the data. Pokhraj Guha analyzed the data. Tapas Kumar Chaudhuri designed the study and analyzed the data.

Acknowledgments

We gratefully acknowledge the support from ICMR – Indian Council of Medical Research, India for providing the project grants (Project sanction no. 54/2/2012-HUM-BMS). I also express my gratitude to the Blood bank of Siliguri district hospital who helped us to collect blood samples from different area of the North Bengal region.

References

[1] A.F. Admetlla, E. Bosch, M. Sikora, T.M. Bonet, A.R. Soriano, A. Muntasell, J. Immunol. 181 (2008) 1315–1322.
[2] S. Agarwal, S.K. Srivastava, M. Borkar, T.K. Chaudhuri, Tissue Antigens 72 (2008) 120–130.
[3] S. Akira, K. Takeda, Nat. Immunol. 4 (2004) 499–511.
[4] M. Bamshad, T. Kivisild, W.S. Watkins, M.E. Dixon, C.E. Ricker, B.B. Rao, J.M. Naidu, B.R. Prasad, P.G. Reddy, A. Rasanayagam, S.S. Papiha, Genome Res. 11 (2001) 994–1004.
[5] L.B. Barreiro, L. Quintana-Murci, Nat. Rev. Genet. 11 (2010) 17–30.
[6] M. Debnath, T.K. Chaudhuri, Int. J. Hum. Genet. 6 (2006) 159–162.
[7] M. Eaaswarkhanth, B. Dubey, P.R. Meganathan, Z. Ravesh, F. A. Khan, L. Singh, K. Thangaraj, J. Hum. Genet. 54 (2009) 340–348.
[8] W. Eder, W. Klimecki, L. Yu, E. Von Mutius, J. Riedler, C. Braun-Fahrländer, D. Nowak, F.D. MartinezALEX study team, J. Allergy Clin. Immunol. 113 (2004) 482–548.
[9] P. Guha, S. Bhattacharjee, C. Nayak, T.K. Chaudhuri, Hum. Immunol. 74 (2013) 673–680.
[10] P. Guha, A. Das, S. Dutta, S. Bhattacharjee, T.K. Chaudhuri, Hum. Immunol. 76 (2015) 789–794.
[11] A.D. Gupta, Anthropology, University of North Bengal, India, 2012.
[12] S. Krupanidhi, Y.R. Ahuja, B. Carani, WMC 2 (2011) 1–15.
[13] H. Laayouni, M. Oosting, P. Luisi, M. Ioana, S. Alonso, I.R. Ponce, PNAS 111 (2014) 2668–2673.
[14] B.B. Mondal, T.K. Chaudhuri, Recent Adv. Anim. Sci. Res. 1 (2000) 121–125.
[15] M.G. Netea, C. Wijmenga, L.A. O'Neill, Nat. Immunol. 13 (2012) 535–542.
[16] D. Reich, K. Thangaraj, N. Patterson, A.L. Price, L. Singh, Nature 461 (2009) 489–494.
[17] N.J. Risch, Nature 405 (2000) 847–856.
[18] K.K. Shrestha, Occas. Pap. Sociol. Anthropol. 11 (2009) 38–47.
[19] A.M. Sutherland, M. Ainsley, D.N. Cook, Clin. Infect. Dis. 41 (2005) 403–407.
[20] R. Tamang, L. Singh, K. Thangaraj, J. Biosci. 37 (2012) 911–919.