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3 Detection of HLA class II alleles in the Muslim population of South India

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Abstract

*The present study has investigated on HLA class II -DQB1 gene in population of Srirangapatna at the southern region of India. 42 individuals in comparison with their most homologous alleles have shown new nucleotide substitutions which lead to new alleles. 22.2% of new alleles comprised only one or two nucleotide variations. DQB1*03 is the most frequent allele group in the present study which has displayed by 20 (44.5%) samples and allele group DQB1*02 with six samples (13.33%) as the lowest frequency. Allele DQB1*03:23:01 was demonstrated as most frequent allele by 18 (40%) samples. We identified 87% of alleles represent at least one non-synonymous mutation while 6% of alleles having only synonymous nucleotide variations. According to numerous ethnicities in India, high-resolution HLA typing is essential to figure out new HLA alleles which have not detected so far through classical approaches.*

Keywords: *HLA DNA sequence, Anthropology, South India, New alleles*

Introduction

HLA genes polymorphism in the world populations have been extensively investigated as an informative markers to assessing human population's diversity basis on anthropological point of view (Buhler and Sanchez-Mazas 2011) and also is in use as a genetic marker to track of some autoimmune related disease in the clinical context. HLA gene allelic diversity have been interpreted as a classical approach by the most earlier investigations to demonstrate similarity and diversity within and between human populations while the present study has only attempted to identify existing or potential alleles in target population regardless displaying population relationship and phylogenetics aspects.

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Material and Methods

Population and location

Population under the study inhabited in Srirangapatna which is located near the city of Mysore in Karnataka state at the Southern region of India. Srirangapatna town 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, Mandya and Maddur town.

Samples

Blood samples collected from 45 unrelated individuals randomly after obtaining written consent which approved by the ethical committee of University of Mysore. 5 ml blood collected from each participant and stored in ethylenediamine tetraacetic acid (EDTA) Vacutainer®1 tubes at 4 degrees Centigrade (ºC).

DNA Extraction and Genotyping

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

Statistical Analysis

All nucleotide variations were identified by using SeqScape software V2.5 (Applied Biosystems) to detect synonyms and non-synonymous substitution and subsequently amino acid sequence alteration 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. All sample DNA sequences were aligned based on HLA-DQB1 reference sequence available in IPD-IMGT/HLA Database. To determine the most homologous allele, each sample DNA sequences were aligned with the existing allele DNA sequences of related locus on IMGT/HLA Database were defined in last Release (3.35.0) of the database (Robinson et al., 2015).

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Results and discussions

Number of new alleles in human leukocyte antigen (HLA) has considerable growing in per IMGT/HLA Database updating report. Development of HLA genotyping platforms facilitated the detection of new alleles in the world populations as the expansion of 10,533 HLA alleles which have been reported on January 2014 to more than 13000 alleles in 2015 and to 21,499 alleles in the last release of the database (3.35.0) on January 2019 (Robinson et al., 2015). The present study has been tried to identifying the existing and possible potential alleles in the Muslim population of Srirangapatna who inhabited in Karnataka state at the southern region of India.

HLA-DQB1 and other HLA class II allele diversities have been considered only at exon 2 in previous studies as a conventional approach (Hernandez‐Frederick(a) et al., 2014). DNA sequence variations observed in comparison with its most homologous allele to detecting nucleotide substitutions at the respective codon positions (Table 1). Existing two identical alleles with complete match i.e. without change in nucleotide sequences were observed in three samples that one of them is belong to DQB1*03:01:02, and two with DQB1*03:23:01. Ten alleles (22.2%) comprised one or two nucleotide variations. Three nucleotide variations from respective most homologous allele were observed in DQB1*03:01:02, *03:23:01, *05:64, *06:51:01-02 and *06:79:01 alleles. Alleles DQB1*03:23:01, DQB1*05:43:01 and DQB1*06:51:01 have been demonstrated in more than one individuals in the population, i.e. similar pattern of nucleotide substitutions carried by two or three individuals (Table 1). The most frequent allele group in the present study is DQB1*03 which displayed by 20 (44.5%) samples while allele group DQB1*02 with six samples (13.33%) were indicated as lowest frequency within population under the study. Allele DQB1*03:23:01 was demonstrated as most frequent allele in this population which is represented by 18 (40%) samples (Table 2). Frequency of alleles at each allele group with respective position codon changes have been described in table 2. Subsequent analysis of the nucleotide diversities has demonstrated 87% of potential alleles in the population carrying at least one non-synonymous mutation while 6% of alleles having only synonymous nucleotide variations (Fig. 1). Polymorphic sites within exon2 were illustrated according to HLA-DQB1 reference sequence in figure 2. Nucleotide variations in HLA-DQB1 gene and other class II genes such as HLA-DPB1, distributed evenly along exon 2 which has been displayed in previous investigations (Hernandez‐Frederick et al., 2016; Hernandez‐Frederick(a-b) et al., 2014). Although nucleotide variations along codon positions in exon 2 distributed evenly in 34 codon positions in the present study and no variations were found in

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remains positions, nucleotide substitutions in codons 100, 196 and 238, were demonstrated highest frequency, respectively with 32, 39 and 45 (Fig. 2). The high nucleotide diversity reflects the extreme polymorphic nature of HLA genes in genome (Parham et al., 1995; Hernandez - Frederick (b) et al., 2014).

Conclusion

We identified 42 HLA class II (DQB1) alleles in Muslim population in Srirangapatna area. Two alleles have been detected in the population with homologous equivalents without nucleotide substitutions and ten alleles (22.2%) comprised not only one or two nucleotide variations also lead to amino acid sequence alteration when compared to their most homologous neighbor alleles in IMGT Database. Due to extensive ethnic diversity in Indian population and growing new allele's apparition in different world geographical area, the high-resolution HLA typing in Indian ethnic communities is essential to figure out new HLA alleles which have not detected so far through classical approaches (Gowda et al., 2016). Recent advances in high-resolution HLA genotyping (Cereb et al., 2015; Lange et al., 2014) will not only improve the identification of new HLA alleles but also help to discover new features in previously described HLA alleles (Hernández-Frederick et al., 2016).

Ethics approval and consent to participate

The Ethical Committee of the Mysore University in India approved the ethical clearance of the study. Written consent was provided from all participants.

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Competing interests

The authors declare that they have no competing interests.

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Most homologous neighbor in IMGT Database	NV	Frequency	Codon change & Position	AA change	Type of mutation	InDel(deletion or insertion) /SNP
DQB1*03:01:02	3	1	**122**GGG>GCG	G41A	Non-synonymous	Missense
			193GGG>CGG	G65R	"	"
			210CGG>CGT	-	Synonymous	Silent
DQB1*03:23:01	3	2	**100**CGC>TGC	R34C	Non-synonymous	Missense
			207CGG>CGT	-	Synonymous	Silent
			218CGG>CAG	R73Q	Non-synonymous	Missense
DQB1*03:23:01	1	2	**202**GGG>CGG	G68R	Non-synonymous	Missense
DQB1*03:23:01	3	1	**105**GCT>GCC	-	Synonymous	Silent
			134CGG>CAG	R45Q	Non-synonymous	Missense
			207CGG>CGT	-	Synonymous	Silent
DQB1*03:23:01	2	1	**105**GCT>GCC	-	Synonymous	Silent
			202GGG>CGG	G68R	Non-synonymous	Missense
DQB1*03:23:01	3	1	**105**GCT>GCC	-	Synonymous	Silent
			151GGC>TGC	G51C	Non-synonymous	Missense
			202GGG>CGG	G68R	"	"
DQB1*03:23:01	1	1	**156**CTG>CTA	-	Synonymous	Silent
DQB1*03:23:01	3	1	**105**GCT>GCC	-	Synonymous	Silent
			156CTG>CTA	-	"	"
			161CCG>CGG	P54R	Non-synonymous	Missense
DQB1*05:03:14	2	1	**158**ACG>ATG	T53M	Non-synonymous	Missense
			237ACG>ACC	-	Synonymous	Silent
DQB1*05:43:01	1	2	**237**ACG>ACC	-	Synonymous	Silent
DQB1*05:43:02	2	1	**184**CAC>TAC	H62Y	Non-synonymous	Missense
			243GCG>GCA	-	Synonymous	Silent
DQB1*05:64	3	1	**128**ATC>ACC	I43T	Non-synonymous	Missense
			131GGG>GCG	G44A	"	"
			237ACG>ACC	-	Synonymous	Silent
DQB1*06:51:01	2	2	**100**CGC>TGC	R34C	Non-synonymous	Missense
			237ACG>ACC	-	Synonymous	Silent
DQB1*06:51:01	3	1	**100**CGC>TGC	R34C	Non-synonymous	Missense
			156CTG>CTA	-	Synonymous	Silent
			237ACG>ACC	-	"	"
DQB1*06:51:01	3	1	**100**CGC>TGC	R34C	Non-synonymous	Missense
			218CGG>CAG	R73Q	"	"
			237ACG>ACC	-	Synonymous	Silent
DQB1*06:51:02	3	1	**100**CGC>TGC	R34C	Non-synonymous	Missense
			203GGG>GAG	G68E	"	"
			237ACG>ACC	-	Synonymous	Silent
DQB1*06:79:01	3	1	**207**CGG>CGT	-	Synonymous	Silent
			208AGT>CGT	S70R	Non-synonymous	Missense
			237ACG>ACC	-	Synonymous	Silent

Table1. Description of identified potential alleles in individual samples

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with new nucleotide variations (alleles comprised with more than 3 substitutions not demonstrated in the table).
NV, number of nucleotide variations between novel and homologous allele.
AA change, amino acid change
Nucleotide position and change(s) are given in bold.

Allele group	Homologous Allele	Codon change & Position	Sample Frequency	Percentage
DQB1*02	DQB1*02:19	76-92-96-97-119-120-121-210-131	2	4.444%
	DQB1*02:39	76-92-96-97-121-124-131-200-202-210	4	8.888%
DQB1*03	DQB1*03:01:02	122-193-210	2	4.444%
	DQB1*03:23:01	96-100-105-121-134-141-151-154-156-161-169-186-198-199-202-203-207-208-218	18	40%
DQB1*05	DQB1*05:03:14	158-237	1	2.222%
	DQB1*05:43:01	237	2	4.444%
	DQB1*05:43:02	184-243	1	2.222%
	DQB1*05:64	121-128-131-210-237	2	4.444%
	DQB1*05:69	105-131-151-157-171-210-237	1	2.222%
	DQB1*05:141	131-154-157-169-218-237	1	2.222%
DQB1*06	DQB1*06:51:01	100-105-141-154-156-161-169-207-218-237	6	13.333%
	DQB1*06:51:02	100-134-151-154-156-169-203-207-237	3	6.666%
	DQB1*06:79:01	207-208-237	1	2.222%
	DQB1*06:201	100-134-195-210-237	1	2.222%
Total			45	

Table2. Frequencies of alleles at each allele group

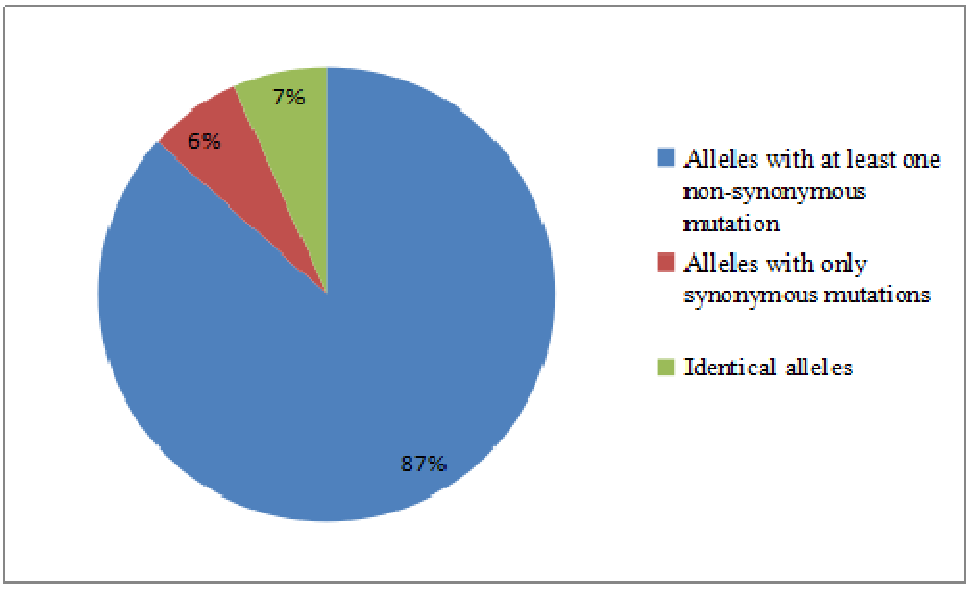

Figure1. Percentage of alleles according to type of mutation

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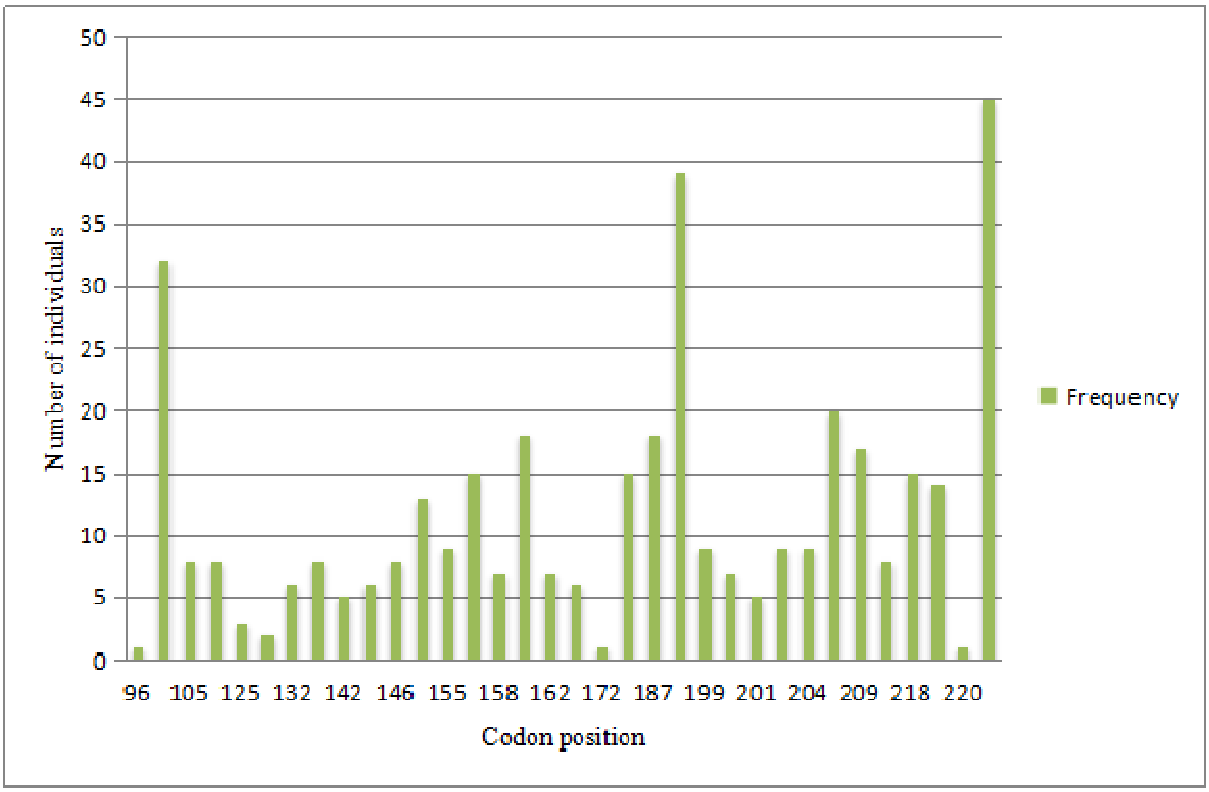

Figure2. Polymorphic sites illustration within exon2. Nucleotide variations along codon positions in exon 2 of HLA-DQB1.

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