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A study of HLA Alleles and Haplotypes associated with type I diabetes mellitus in South India

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Abstract

Background: T1DM is an abominable auto immune disease among children that emerged from the interaction between the environmental and genetic factors coupled with the HLA gene. The major susceptibly genes for T1DM are located in the HLA region present on the 6p chromosome. The objectives of this study are two fold, they are; • To conduct a pilot case control study to examine the association of HLA alleles and T1DM in people from South India • To determine the specific HLA DR-DQ two-locus haplotype of T1DM in South India.

Materials and Methods: Of 118 children screened, 57 South Indian unrelated children with T1DM who were attending the pediatric diabetic endocrinology outpatient clinic of Kovai Medical Centre and Hospital, Coimbatore were consecutively included in the study. The control normal subject group include 57 normal healthy people which did not have any T1DM or any autoimmune disorder. HLA DRB1*/HLADQB1* antigens were determined by the PCR with sequence specific primers (PCR-SSP). Statistical analysis of haplotype results was done depending on the logistic regression model.

Results: The following were considered as significant allelic and haplotethic associations with T1DM: DRB103 (p < 0. 001), DRB104 (p = 0. The current study indicated that the following alleles an haplotypes were significant telecoms with T1DM College Admissions Essay Prompts; * DRB103 –0. Just the haplotype DRB103-DQB106 was found in five cases and p value was significant. Positive association was detected for alleles DRB107 (Chi-square = 26. 02; p < 0. 001), DRB114 (Chi-square = 3. 62; p = 0. 031), DQB10301, DQB10304, DQB10302 (Chi-square = 62. 34; p < 0. 001), DQB105 (Chi-square = 10. 52; p)

Conclusion: In this study we are able to establish that the interaction between specific HLA alleles and T1DM is considerable in more than one way. Specific genotypes such as, DRB103-DQB102 combination are very much responsible for the risk than any other alleles of DRB1 and DQB1. Others alleles which include DRB115-DQB106, DRB115-DQB105 and DRB107-DQB102 are given with a lower risk. Some of these gene alleles can be utilise as diagnostic markers of T1DM.

Key Words: Type 1 Diabetes Mellitus; Autoimmune diseases; Haplotypes.

Introduction

T1DM is the irreversible loss of insulin producing beta cells in the islets of Langerhans due to autoimmunity. This process is genotypically determined and is precipitated by one or many environmental stimuli. It generally evolves over several months to years, possibly decades, while

the patient is still normoglycemic and without symptoms. While auto antibodies are not identified until the process of autoimmunity is already active, auto genes are already inherited at birth. Biochemical indices for the metabolic disorder can be measured once considerable destruction of beta cells but before clinical hyperglycemia. This long latent period shows the large mass of functioning beta cells that are needed to be destroyed before hyperglycemia becomes symptomatic.

Currently, India has the majority of the children affected by T1DM in South-East Asia. Per 100,000 children between aged 0–14 years of age, India has 3 new cases of T1DM based on the 6th edition of the International Diabetes Federation Diabetes Atlas. The prevalence of diabetes in India varies: Several studies document that ladies assert 17. The number of cases with respect to children in Karnataka was estimated to be 93 per 100,000 children, 3. Two reports showed Chennai to have 2 cases per 100,000 children, and 10. Karnal (Haryana) 2 per 100000 children 24/05/2012. Nevertheless, T1DM is relatively frequent and widespread, unlike the fluctuation in results that characterizes this disease. T1DM is also on the rise at the rate of 3–5% per annum, though not as steep as T2DM. It confirmed that T1DM has two modes, which are children of age between four and six years and adolescents aged between ten and fourteen years. Totally, approximately 45% of children have the T1DM diagnosed at an age of 10 years or younger. T1DM does not particularly favor any gender, and thus its manifestations are not inclined to be seen in a particular sex.

MHC is an acronym in human and animals that stands for major histocompatibility complex that is a collection of genes that produce a variety of cell markers, antigens, and additional proteins that are concerned with immune responsiveness. Such close association that the human leukocyte antigen (HLA) typing is being referred to as human MHC.

HLA loci are one of the most polymorphic regions in the human genome and the genetic data originating from this site are highly informative in numerous disease association and population differentiation investigations. New developments in DNA typing regarding the HLA genes make it possible to obtain more specific information on these genes, on the molecular pathology of diseases and the evolution of nations in India. Also, when the extended haplotypes have been analyzed, they can help in the identification of some population structures and relations that

would not be noticed when working with the individual loci. Specifically within the HLA region LD is very high which permits the comparison of population divergence based on haplotypic data along with single locus analysis. While DNA typing is far more comprehensive than serology, it has uncovered specific alleles and sequence motifs mainly in DRB1 and DQB1 loci, which are firmly linked with T1DM.

This present research work is planned to elaborate the association between HLA alleles and T1DM in the context of South Indian population.

Materials and Methods

Thus, the primary purpose of this research is to assess the relationship between diabetes type 1 with HLA alleles /haplotypes. It is a prospective type of observational study, which is expected to be conducted among the patients of the Kovai Medical Centre and Hospital, Coimbatore. With reference to the given observation of retrospective data, in one year time period, 185 patients were registered under the outpatient record and the proportion of likelihood for below 18 years age group is 5% of total number of patients. According to the criteria of inclusion and exclusion all the patients were chosen. The present study consisted on patients with type I DM of less than 18 years of age, diagnosed clinically and laboratory investigations.

DNA extraction from whole blood

Peripheral blood was obtained from the index finger prick and DNA was extracted from 3 ml of the collected blood by the salting out method as described by Syed (2008) . PBS, DMEM and all other stock solutions, except SDS, was autoclaved at 120ºC and 15 lb pressure for 30 min and stored on ice then at 4ºC.

To be precise, 3000 micro litre of peripheral vein blood was added to 12000 micro litre of Red Cell Lysis Buffer (RCLB) inside a 15 micro litre polypropylene centrifuge tube. The tube was then turned on end and allows to stand for 5 minutes to enhance the lysis of red blood cells. It was then centrifuged at 2000 rpm for 10 minutes. The liquid on the top was removed, and then the solid on the bottom was collected. After that this pellet was washed with 15ml RCLB and the sample was centrifuges at 2000rpm for 10 minutes. The clear nucleated cells (White Blood Cells,

WBCs) have sedimented at the bottom and were lysed with 3 ml of Nuclear Lysis Buffer (NLB) and Sodium Dodecyl Sulphate (SDS) thoroughly before the next step.

To this mixture to which 1 ml of 6M Sodium Chloride (NaCl) was added, the proteins will precipitate. The cultures were next mixed and the samples were vortexed before adding 2 ml of chloroform and shaking it well. This caused the formation of a rather uniform, milky suspension, which was spun for 10 minutes at 2000 RPM. The DNA which is in soluble form was in the aqueous phase while the protein layer, also known as the interphase, was not disturbed into a new sterile 15 ml centrifuge tube. Two volume equivalents of 15% ethanol were then added to it and the tube was shaken several times (slowly) to from the DNA precipitate. Subsequently the precipitated DNA was transferred to 1. 5 ml microcentrifuge tube containing 70% ethanol and the sample spun at 2000 rpm for 5 minutes. To eliminate as many proteins and salts as possible from the DNA the alcohol washing step was performed again. The alcohol was then decanted, after which the DNA was washed in 200µl of Tris-EDNA (TE) buffer.

Quality and quantity checking of DNA

Optical Density Method

DNA concentration and the absence of proteins and carbohydrates in purified DNA was assessed form the OD of DNA at 260 nm using UV spectrophotometer. It is significant OD of 1 at 260 nm equal to 50 ng/ul of double stranded DNA (Sambrook et al. , 1989)[18]. DNA was checked for its purity by scanning absorbance at 260, 280nm in UV spectrophotometer. The value of the ratio =OD at 260 nm /OD at 280 nm was determined. DNA with the ratio of 1. The latter was divided into 8 which was used for further analytical work. DNA concentration was analyzed by applying the following calculation:DNA concentration was analyzed by applying the following calculation:

This will be represented as the concentration of DNA = OD 260 × Dilution Factor × 50 ng/µl

HLA-DRB1* typing by PCR-SSP

PCR –SSP technique of genotyping was used for HLA- DRB 1* with reference to Olerup & Zitterquist (1993) [19]. The internal control primer for human growth hormone gene was added in all reactions to exclude PCR failure of individuals and therefore to prevent wrong allele

calling. First, PCR reactions contains of 5. 2 µl double distilled water, 1. An additional 2 ul of 10 x PCR buffer, 0. 5 ml of 5% glycerol, 0. Thus for the dNTP solution, 2 µl of 10 mM each of dATP, dCTP, dGTP and dTTP was used. Final concentrations in the reaction mix are 1 ul of 5 U/ µl of Taq DNA Polymerase enzyme, 0. 4 ml of cresol red, 0. 3 ul of 10mm forward and reverse primers and 4 ul of DNA. For the PCR cycles which were used for this HLA-DRB1*, two rounds of PCR with two different annealing temperatures were employed. The reaction included initial denaturation at 94ºC for 2 minutes, followed by a touchdown PCR program where there was 10 cycles of denaturation at 94ºC for 10 seconds, annealing at 64ºC for one minute, then another 20 cycles at 94ºC for 10 seconds, annealing at 61ºC for 50 seconds before extension at 72ºC for 30 sec

HLA – DQB1* typing by PCR – SSP

PCR – SSP (sequence specific primer) technique of genotyping of the HLA – DQB1* was done in the present investigation as described by Bunce et al. (1995) [20]. The PCR experimental set up as follows; typing per sample for DQB1* alle of 9 primer mixes (Occimum Biosolutions, Hyderabad, India) were prepared adding 2µM of allele specific 0. Common control at 5µM of internal control primer and 1µg of cresol red in the reaction. Thus, in all the reactions to check the alone PCR failures, internal control primer for human being growth hormone gene was used in internal control. Primer adds were prepared by spott ing PCR tubes and placed in the -20 °C freezer. 9 PCR adds for 9 reactions including the negative control are as follows: 18. , 72 µl of double distilled water was added and it was 11. 7 µl of 10 x PCR buffer (Final conc. 1x), 5. 64 µl 100% glycerol, 2. 7 µl 10 mM dNTP mix and 22 µl of template DNA (100 ng /reaction). Primer predotted PCR reaction tubes were mixed with the PCR mix incorporating into each tube. Therefore each reaction includes 7µl of PCR solution, 5µl of primers and 2µl of the DNA template PCR.

Detection of PCR products by Electroporesis method

The PCR products were identified by electrophoresing, 12 µl of the PCR product in a 1.5% agarose gel (Sambrook *et al.,* 1989) [18]. The gel platforms and gel combs were cleaned with 70% alcohol. The platform was placed on a balancing table and the combs were set in place. 1.05 g of agarose (1.5%) was added to 70 ml of Tris-Boric Acid- EDTA (TBE) buffer and the solution

was boiled in a microwave oven. It was allowed to cool at 60º C and 6 µl of 10 µg / ml Ethidium Bromide was add up, mixed and poured into the platform. It was let to solidify for 20 minutes and the gel was immersed in cold 1× TBE buffer in the electrophoresis tank. After another 10 minutes the combs were removed with care. The PCR products were mixed with the loading dye, filled into the wells and electrophoressed at 100 volts for 20 minutes. After electrophoresis, the gel was envisaging under ultra-violet illumination and recorded in a gel documentation unit. Alleles were allocated according to the pattern of band formation.

Statistical Analysis

The data collected from the patients and controls are analysed using SPSS version 16 for windows. Descriptive analysis was carried out to exhibit the frequency observation mean and standard deviation. Allelic frequencies were calculated by using direct counting method and the risk relationship for each allele was examined by contrast the allelic frequencies between patients and controls. The odds ratio OR, confidence intervals and p values were calculated by the chi-square test. Logistic regression analysis was used to measure the degree of relationship among measurement variables and nominal variables. The statistical significance is tested at 5% level of significance. The Pearson chi –square analysis results depicts p-value is 0.101, which is better than the level of significance 0.05, hence the null hypothesis is accepted (not significance).

Results

Age wise distribution of cases and controls

In the present study the total numbers of cases enrolled were 57 and controls 57. Distribution of above sample size according to age wise specified in the table 1. Large number of type 1 DM patients are present in >10yrs in both cases and controls when compared to < 10 years.

Table 1. Age distribution of cases and controls

Age	CASES	CONTROL

	COUNT	PERCENT	COUNT	PERCENT
<10 YRS	**13**	**22.8%**	**5**	**8.8%**
>10 YRS	**44**	**77.2%**	**52**	**91.2%**
TOTAL	**57**	**100.0%**	**57**	**100.0%**

Distribution of HLA DRB1* Alleles

A contrast of the frequency of pertinent HLA DRB1* alleles in patients and controls is given in Table 2 with the respective p values in Table 3. Among the HLA class II alleles of the HLA DRB1 locus showed that DRB1*03 is the most frequent allele in patients compared with controls (31.6%vs12.28%) with a (OR-3.297, p value of 0.000), followed by DRB1*04 (cases 22.81%vs controls9.65%) (OR-2.767 &p value 0.007).

A statistically reduced significant in the frequency of DRB1*07 is noticed among patients (cases 2.63% vs controls 21.05%) and p value of 0.000, followed by DRB1*14 (cases 0.88%vs controls 6.14%) (p value 0.031).

The allele DRB1*10 is equally distributed in cases and controls (14.04%vs 11.4%), with a p value of >0.05. Similarly the frequency of DRB1*15 is more among controls when compared with cases (cases 18.42% vs. controls 24.56%), with a p value of 0.259 (>0.05).

Table 2. Frequency of HLA-DR in cases and controls

HLA-DR	CASES		CONTROL	
	COUNT	**PERCENT**	**COUNT**	**PERCENT**
HLA-DRB1*01	2	1.8%	2	1.8%
HLA-DRB1*03	36	31.6%	14	12.3%

HLA-DRB1*04	26	22.8%	11	9.6%
HLA-DRB1*07	3	2.6%	24	21.1%
HLA-DRB1*08	4	3.5%	4	3.5%
HLA-DRB1*09	0	0.0%	1	0.9%
HLA-DRB1*10	16	14.0%	13	11.4%
HLA-DRB1*11	0	0.0%	2	1.8%
HLA-DRB1*12	3	2.6%	5	4.4%
HLA-DRB1*13	2	1.8%	3	2.6%
HLA-DRB1*14	1	0.9%	7	6.1%
HLA-DRB1*15	21	18.4%	28	24.6%
TOTAL	**114**	**100.0%**	**114**	**100.0%**

Table 3. Frequency distribution of HLA-DRB1* alleles among cases and controls

HLA-DR	**Cases (n=57)**	**Control (n=57)**	**OR**	**Low-CI**	**High-CI**	**$\chi 2$**	**p-value**
DRB1*01	2 (1.75%)	2 (1.75%)	1.000	0.138	7.224	0.000	1.000
DRB1*03	36 (31.58%)	14 (12.28%)	3.297	1.662	6.538	12.399	0.000
DRB1*04	26 (22.81%)	11 (9.65%)	2.767	1.294	5.917	7.259	0.007
DRB1*07	3 (2.63%)	24	0.101	0.030	0.347	18.527	0.000

		(21.05%)					
DRB1*08	4 (3.51%)	4 (3.51%)	1.000	0.244	4.100	0.000	1.000
DRB1*09	0 (0%)	1 (88%)	0.000	1.763	2.289	1.004	0.316
DRB1*10	16 (14.04%)	13 (11.4%)	1.268	0.580	2.775	0.356	0.551
DRB1*11	0 (0%)	2 (1.75%)	0.000	1.769	2.302	2.018	0.155
DRB1*12	3 (2.63%)	5 (4.39%)	0.589	0.137	2.526	0.518	0.472
DRB1*13	2 (1.75%)	3 (2.63%)	0.661	0.108	4.031	0.204	0.651
DRB1*14	1 (0.88%)	7 (6.14%)	0.135	0.016	1.118	4.664	0.031
DRB1*15	21 (18.42%)	28 (24.56%)	0.694	0.367	1.312	1.274	0.259

Distribution of HLA DQB1* Alleles

The distribution of HLA DQ alleles is shown in figure 1 with the respective p values in Table 4.

The allelic distribution of HLA DQB1*02 is more in patients when contrast to controls (cases 41.23% vs. controls 15.79 %) (OR 3.741 & p value 0.000). Though the frequency of HLADQB1*06 is more in cases when compared to controls(cases 37.32% vs. controls 28.07%), the p value is 0.121(>0.05).

The frequency distribution of HLA DQB1*0301, 0304(DQ7) & DQB1*0302 (DQ8) taken as DQB1*03 are seen only among controls 14.91% (p value 0.000), followed by DQB1*05 (cases 20.18% vs. controls 38.60%) with a significant p value of 0.002.

No significant difference in the distribution of various other DRB1*and DQB1* alleles is observed among cases and controls.

Figure 1. Distribution of HLA DQ alleles in cases and control group

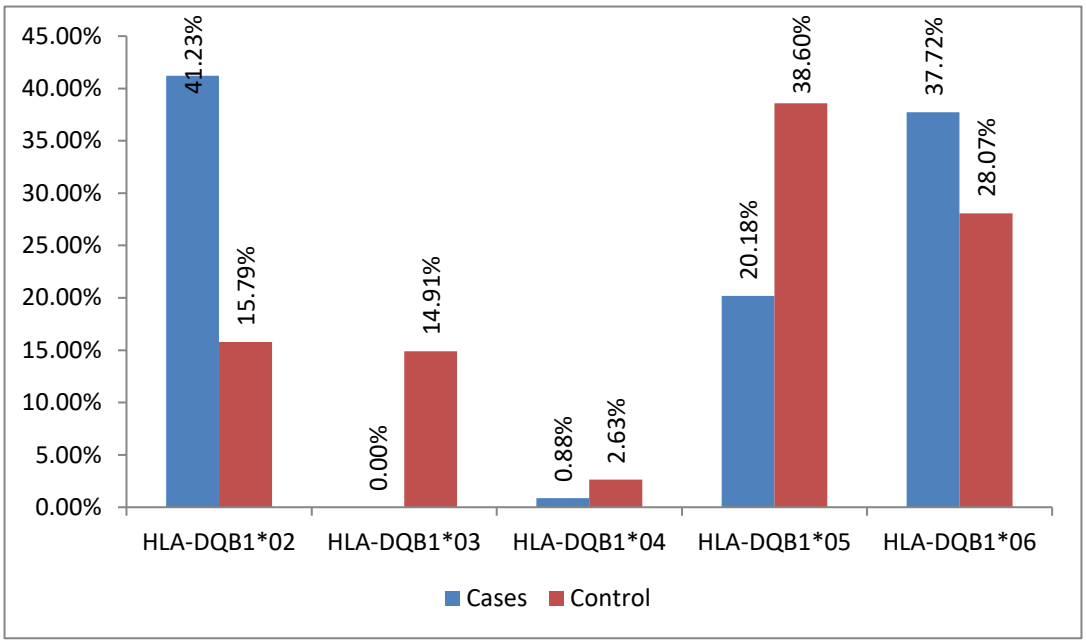

Table 4. Distribution of HLA-DQ alleles in South Indian Type 1 diabetes mellitus patients

HLA-DQ	Cases (n=57)	Control (n=57)	OR	Low-CI	High-CI	χ2	p-value
DQB1*02	47 (41.23%)	18 (15.79%)	3.741	2.000	7.000	18.098	0.000
DQB1*03	0 (0.00%)	17 (14.91%)	0.000	1.879	2.518	18.370	0.000
DQB1*04	1 (0.88%)	3 (26.3%)	0.327	0.034	3.196	1.018	0.313
DQB1*05	23 (20.18%)	44 (38.60%)	0.402	0.222	0.727	9.321	0.002
DQB1*06	43 (37.72%)	32 (28.07%)	1.552	0.889	2.709	2.404	0.121

Distribution of DRB1*-DQB1* two locus haplotype in Type 1 DM Patients:

The frequency of DRB1*03-DQB1*02 haplotype is raised among cases when compared to controls (cases 24.56% vs. controls 4.39%) with a OR -7.098 and p value 0.000. The haplotype DRB1*03-DQB1*06 is present only in cases with a significant p value of 0.024.(Table 5)

The haplotype DRB1*07-DQB1*02 frequency is increased in controls as contrast to cases (cases 2.63% vs. controls 8.77%) with a significant p value of 0.046.($p < 0.05$). The haplotype DRB1*15-DQB1*06 has the highest percentage of distribution among controls (cases 2.63% vs. controls 26.32%) with a p value 0.000. Similarly the haplotype DRB1*15-DQB1*05 is highest in controls (cases 0.88% vs controls 6.14%) with a p value 0.031 ($p < 0.05$).

Table 5. Haplotype frequency in South Indian type 1 diabetes patients

HLA-DR*DQ	Cases (n=57)	Control (n=57)	OR	Low-CI	High-CI	$\chi 2$	p-value
DRB1*01 - DQB1*02	2 (1.75%)	0 (0%)	0.000	1.769	2.302	2.018	0.155
DRB1*01 - DQB1*05	2 (1.75%)	0 (0%)	0.000	1.769	2.302	2.018	0.155
DRB1*03 - DQB1*02	28 (24.56%)	5 (4.39%)	7.098	2.630	19.153	18.743	0.000
DRB1*03 - DQB1*03	0 (0%)	1 (0.88%)	0.000	1.763	2.289	1.004	0.316
DRB1*03 - DQB1*04	1 (0.88%)	1 (0.88%)	1.000	0.062	16.184	0.000	1.000

DRB1*03 - DQB1*05	7 (6.14%)	2 (1.75%)	3.664	0.744	18.031	2.892	0.089
DRB1*03 - DQB1*06	5 (4.39%)	0 (0%)	0.000	1.789	2.340	5.112	0.024
DRB1*04 - DQB1*02	8 (7.02%)	2 (1.75%)	4.226	0.877	20.357	3.765	0.052
DRB1*04 - DQB1*03	2 (1.75%)	1 (0.88%)	2.018	0.180	22.571	0.338	0.561
DRB1*04 – DQB1*05	9 (7.89%)	5 (4.39%)	1.867	0.606	5.759	1.218	0.270
DRB1*04 - DQB1*06	8 (7.02%)	2 (1.75%)	4.226	0.877	20.357	3.765	0.052
DRB1*07 - DQB1*02	3 (2.63%)	10 (8.77%)	0.281	0.075	1.050	3.997	0.046
DRB1*07 - DQB1*03	3 (2.63%)	2 (1.75%)	1.514	0.248	9.233	0.204	0.651
DRB1*07 - DQB1*05	1 (0.88%)	5 (4.39%)	0.193	0.022	1.678	2.739	0.098
DRB1*07 - DQB1*06	1 (0.88%)	2 (1.75%)	0.496	0.044	5.543	0.338	0.561
DRB1*08 - DQB1*02	0 (0%)	1 (0.88%)	0.000	1.763	2.289	1.004	0.316

DRB1*08 - DQB1*04	2 (1.75%)	0 (0%)	0.000	1.769	2.302	2.018	0.155
DRB1*08 - DQB1*05	1 (0.88%)	0 (0%)	0.000	1.763	2.289	1.004	0.316
DRB1*08 - DQB1*06	3 (2.63%)	2 (1.75%)	1.514	0.248	9.233	0.204	0.651
DRB1*10 - DQB1*02	0 (0%)	1 (0.88%)	0.000	1.763	2.289	1.004	0.316
DRB1*10 - DQB1*05	7 (6.14%)	11 (9.65%)	0.613	0.229	1.641	0.965	0.326
DRB1*10 - DQB1*06	6 (5.26%)	4 (3.51%)	1.528	0.419	5.565	0.418	0.518
DRB1*11 - DQB1*03	1 (0.88%)	1 (0.88%)	1.000	0.062	16.184	0.000	1.000
DRB1*12 - DQB1*02	1 (0.88%)	2 (1.75%)	0.496	0.044	5.543	0.338	0.561
DRB1*12 - DQB1*05	0 (0%)	3 (2.63%)	0.000	1.776	2.314	3.040	0.081
DRB1*12 - DQB1*06	0 (0%)	2 (1.75%)	0.000	1.769	2.302	2.018	0.155
DRB1*13 - DQB1*05	0 (0%)	1 (0.88%)	0.000	1.763	2.289	1.004	0.316

DRB1*13 - DQB1*06	2 (1.75%)	2 (1.75%)	1.000	0.138	7.224	0.000	1.000
DRB1*14 - DQB1*05	3 (2.63%)	2 (1.75%)	1.514	0.248	9.233	0.204	0.651
DRB1*14 – DQB1*06	1 (0.88%)	2 (1.75%)	0.496	0.044	5.543	0.338	0.561
DRB1*15 - DQB1*02	0 (0%)	2 (1.75%)	0.000	1.769	2.302	2.018	0.155
DRB1*15 - DQB1*03	3 (2.63%)	3 (2.63%)	1.000	0.198	5.062	0.000	1.000
DRB1*15 - DQB1*05	1 (0.88%)	7 (6.14%)	0.135	0.016	1.118	4.664	0.031
DRB1*15 - DQB1*06	3 (2.63%)	30(26.32 %)	0.076	0.022	0.256	25.829	0.000
DRB1*15 - DQB1*06	3 (2.63%)	30(26.32 %)	0.076	0.022	0.256	25.829	0.000

Discussion

T1DM is Non-Genetic Constitutional, Polygenic disease; insulin dependent diabetes mellitus which begins in childhood or adolescence. There are many causative factors of the disease, amongst which is the HLA gene that proves vital to the disease process. Among these, class II human leukocyte antigen (HLA) DQ and DR antigens, and class II HLA DPA1, DPB1 are significant in the susceptibility to T1DM development. As a matter of fact there is a rather frequent phenomenon when protective DRB1* alleles are linked with susceptible DQ alleles, and

in the same time when neutral or protective DQ are associated with susceptible DR alleles. This counterbalancing influence of the susceptible DRB1 with protective DQB1 or the susceptible DQA1 with protective strictly linked DQB1 may affect the incidence of T1DM.

To the best of our knowledge, the present work is the first observational study identifying HLA alleles and haplotypes in relation to T1DM in children of the South Indian population. Thus, the present study concludes that the HLA-DRB1* alleles responsible for the genetic predisposition to T1DM in the children of South India are mainly DRB103 and then DRB104. In accordance with present findings, previous research including T1DM patient populations derived from east and north zones of India have also pointed out that, DRB103 is the most prominent allele associated with T1DM. Despite this observation, a north Indian and Japanese survey showed DRB109 to be a valuable allelic factor triggering T1DM.

Similarly, comparable rates have been reported in other Asian population in which DRB1*04 is the dominant antigen [22]. Thus, within the Caucasians, DRB1*04 is nearly all significant susceptible allele, with the following allele being DRB1*03.

Similarly the protective allele in the order of frequency are DRB1*07>DRB1*14>DRB1*15. The study conducted among Caucasian , Asian [22] and Indian [23] population showed DRB1*15 as the most protective allele followed by DRB1*07. Especially in Indian children the most common protective alleles are DRB1*07 and DRB1*15. In addition DRB1*14 is found to be a protective allele in South Indian children (with a significant p value 0.031).

The order of frequency of susceptible allele among DQB1 (other HLA class II genetic loci) in South Indian children are DQB1*02>DQB1*06. Similar studies conducted in various other parts of India [23] have also described DQB1*02 as almost all significant susceptible allele. Though DQB1*06 have a increased frequency of distribution among cases (37.72%), the p value appears to be >0.05 (p value 0.121).

Similarly the protective alleles are DQB1*05 (p value 0.002), DQB1*0301,0304 and DQB1*0302. But among Caucasians, Finnish [24] and Swedish population DQB1*0302 was expressed as a susceptible allele. Likewise, among Asians DQB1*0302 have been reported as a susceptible allele [22].

The study also revealed the following haplotypes as protective and susceptible. DRB1*03-DQB1*02 the most significant susceptible haplotype. Results are consistent with the haplotype combinations reported in Caucasians, Koreans [25] and North Indians [21]. But DRB1*0405-DQB1*0401 was reported to be the susceptible haplotype among Asians [22].

Distribution of protective haplotype among South Indian children are DRB1*15-DQB1*06, DRB1*15-DQB1*05 and DRB1*07-DQB1*02,which is comparable with results published for Asians, Caucasians and other Indian population.

It is recommended that incidence of diabetes are due to genes. Counter balancing effect linking between susceptible DRB1 and protective DQB1, and vice versa might be an important gene factors accountable for the incidence of Type I DM[26].

Conclusion

In this study, we conclude that the association of specific HLA alleles with Type 1 Diabetes (TID) is remarkable in several aspects: 1. For DRB1 and DQB1, the risk is determined by specific combinations of DRB1 and DQB1 alleles rather than by the genotype for individual loci. 2. Both susceptible and protective, highly significant DR-DQ associations with TID are observed, dependent on the particular DR-DQ haplotype. 3. Multiple haplotypes are positively associated with TID (susceptible), while many haplotypes are negatively associated (protective). Specific genotypic combinations, such as DRB1*03-DQB1*02, are associated with an increased risk. Conversely, haplotypes like DRB1*15-DQB1*06, DRB1*15-DQB1*05, and DRB1*07-DQB1*02 are associated with a decreased risk, acting as protective factors. Furthermore, a case-controlled, multicentric, genetic epidemiological study is highly warranted to dissect the molecular pathology of Type 1 Diabetes in a country like India, where epidemics and endemics are not uncommon. The aforementioned gene alleles could be very helpful as diagnostic tools for Type 1 Diabetes.

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