HLA class II SNP interactions and the association with type 1 diabetes mellitus in Bengali speaking patients of Eastern India

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Abstract

Background: Several studies have demonstrated a fundamental role for the HLA in the susceptibility of, or protection to, type 1 diabetes mellitus (T1DM). However, this has not been adequately studied in Asian Indian populations. To assess the frequency of HLA class II (DPA1, DPB1, DQA1, DQB1 and DRB1) associated to susceptibility or protection to T1DM in a Bengali population of India with diabetes.

Results: Single nucleotide polymorphism study. The HLA genotyping was performed by a polymerase chain reaction followed by their HLA-DP, DQ, and DRB1 genotypes and haplotypes by sequencing method. The results are studied by Plink software. The χ² tests were used for the inferential statistics. To our knowledge, this study is the first of a kind which has attempted to check the HLA association with T1DM by SNPs analysis. The study recruited 151 patients with T1DM and same number of ethno-linguistic, sex matched non-diabetic controls. The present study found a significant SNP rs7990 of HLA-DQA1 (p = 0.009) negative correlation, again indicating that risk from HLA is considerably more with T1DM.

Conclusions: This study demonstrates that the HLA class-II alleles play a major role in genetic basis of T1DM.

Keywords: HLA, T1DM, SNP, India, Haplotyping, Alleles

Background

Type 1 diabetes mellitus (T1DM) (OMIM-222100), results from a cellular-mediated autoimmune destruction of the Beta-cell of the pancreas [1]. T1DM is a disease of major public health concern [2-4]. The previous studies showed that in India, the prevalence of T1DM varies from about 1.6 to 10.5/100000/year [5,6]. The epidemiological study conducted in South Indian population for four years, suggested the prevalence of T1DM in India is increasing. The overall prevalence of T1DM in Karnal district, a North Indian city with a population of 222017, is 10.20/100,000 population [7].

The genetic risk factors of T1DM are better understood than the environmental risk factors. Studies on Human and animal models show the MHC class II-mediated effects on the disease susceptibility [8]. Early studies identified in the Human Leukocyte Antigen (HLA) genes, located on chromosome 6p21.31 as T1DM susceptibility genes. Resulting studies showed an association between the insulin gene on chromosome 11p15.5. The risk of T1DM is linked with about 18 regions of the genome. These regions, each of which may contain multiple genes, labeled IDDM1 to IDDM18. The best studied is IDDM1, which contains the HLA genes encode proteins of the immune response [9,10].

HLA is one of the most polymorphic genetic systems in Human genome. IMGT/HLA database have reported 6,275 HLA alleles [11]. Because the HLA class II molecules are polymorphic, they can embrace a wide variety of antigens in their antigen-binding groove and present them to diverse T-lymphocyte antigen receptors, triggering antigen recognition. Several studies have displayed HLA class II alleles, DQ and DR influence T1DM susceptibility. The contribution of the DQ
molecules to overall disease susceptibility might be
genotype dependent and/or may be influenced by the
DRB1*04 allele on the haplotype [12].

The HLA-DQ heterodimers encoded by the
DQA1*0301, DQB1*0302 and DQA1*0501, DQB1*0201
alleles have the strongest association with T1DM [13].
These alleles are in LD with the HLA-DR4 and -DR3
alleles. In T1DM at least one allele of DR3 or DR4 is
found in 95% in Europeans, and individuals with both
DR3 and DR4 are particularly susceptible to T1DM,
whereas, the DR2 allele is protective [14–16].

In North Indian T1DM patients also, the homozygos-
ity and heterozygosity of DRB1*0301 and DRB1*04
alleles is significantly associated [17–19]. The Indian
samples show HLA-C*0702 allele and shared HLA-B
*0801 and DQB1*02 with the European 8.1AH [20–23].

The role of DP molecules has yet to be resolved satisfac-
torily. The results favor DPB1*0301 and DPB1*0202
alleles as predisposing for T1DM [24]. Analysis of
family-based data from the Human Biological Data
Interchange (HBDI) repository and Italian studies,
suggests the presence of a T1DM protective locus at or
near DPB1*0101 [25]. It is hypothesized that the
strongest candidates for increasing T1DM risk among
DR3-DQB1*0201/DR4-DQB1*0302 individuals is of alleles
of DP and DRB1*04 subtypes and, in particular, the
absence of reportedly protective alleles DPB1*0402
and/or DRB1*0403 [26].

There are some studies on HLA-DP in different ethnic
groups mainly about HLA-DPB1. In a study from Sudan,
there were no significant differences between Sudanese
patient and control groups in HLA-DPB1 frequencies
[27]. Although, there was also no noticeable association
between T1DM and HLA-DPB1 allele in Japanese [28].

From Indian T1DM patients, so far no studies have been
reported on DP molecules.

Although different methods exist to characterize the
polymorphisms in HLA genes, the 12th International
Histocompatibility Workshop suggested the Sequence
Based Typing (SBT) methods [29]. Therefore, we
conducted a hospital-based case–control study in the
West Bengal region of India to find out the role of
HLA- DRB1, DQ and DP gene polymorphisms in pro-
gress T1DM.

### Methods

#### Subjects

In the present study 151 T1DM patients were recruited
from six different hospitals - Calcutta Heart and Re-
search Clinic; Endocrinology Department, Calcutta Me-
dical College & Hospital; Endocrinology Department,
SSKM Hospital; Netaji Subhash Chandra Bose Cancer
Research Institute; Rabindranath Research Institute of
Cardiac Sciences; School of Tropical Medicine; from
metropolitan Kolkata. The inclusion criteria considered
for recruitment of cases were an onset of diabetes below
39 years of age, and presenting with or without acute
ketosis with absolute insulin dependence, as shown by
a deficient C-peptide secretion i.e., a C-peptide value
less than 0.6 (0.6-3.2)ng/ml and antibody positivity
for Glutamic Acid Decarboxylase antigen(GADA) and,
Insulinoma-Associated Protein-2 Antibodies (/IA-2/ICA512)
[30]. Patients of at least one year duration were selected to
exclude acute or “honeymoon” phases.

The sampled subjects speak Bengali. The samples
represented in our study is mainly originated from

### Table 1. List of primers used in the present study to amplify genes

| Designation | Chromosome location | Sequence | Annealing temperature | Product size(bp) |
|-------------|---------------------|----------|-----------------------|-----------------|
| HLA-DPA1*   | 6p21.3 (Exon2)      | F-5: GCGGACCATGTGTTCAACTTAT 3' | 55°C       | 210             |
|             |                     | R-5: GCCTGAGTGTGGTAGGGACG 3'    |            |
| HLA-DPB1*   | 6p21.3 (Exon2)      | F-5: GAGAGTGGCGGCGCTCGTCAT 3'  | 63°C       | 327             |
|             |                     | R-5: GCCGGCCCAAAGCCCCCTACCT 3' |            |
| HLA-DQA1    | 6p21.3* (Exon1)     | F-5: CAACTCTTCAGCTAGTAAC 3'    | 58°C       | 262             |
|             |                     | R-5: CATGCACCTACCCCAAT 3'      |            |
|             | 6p21.3* (Exon2)     | F-5: ATGTTGTAACCTTGACAG 3'     | 55°C       | 229             |
|             |                     | R-5: TTGGTAGCAGGCTTAGGGATG 3'  |            |
|             | 6p21.3* (Exon3)     | F-5: AGGGTCCAGTGACAGTCAAC 3'   | 54°C       | 328             |
|             |                     | R-5: CTGGACAGCAAAAGGAGCCT 3'   |            |
| HLA-DQB1*   | 6p21.3 (Exon2)      | F-5: CATGTGCCTACTTCACCAACG 3'  | 58°C       | 211             |
|             |                     | R-5: CTGGTAGTTGTCGTCAGCAAC 3'  |            |
| HLA-DRB1*   | 6p21.3* (Exon2)     | F-5: CCCACACGAGTTCTTGGTTG 3'   | 60°C       | 274             |
|             |                     | R-5: CCCTTGCACTGTGAAGCTC 3'    |            |

*(Tanaka et al. 1999), *(Cordova et al. 2005).
districts-Kolkata, followed by South-24-Parganas, North24-Parganas, Howrah, Hoogly, comprising a small geographical area and historically forming a cultural zone. West Bengal is the melting pot of Indo-Aryan, Austro, Dravidian, Tibeto-Burman and various other languages [31]. Some scholar stated that Bengal had many striking resemblance with the Dravidian culture [32], where as others suggested that Bengal as the meeting place of Aryan, non-Aryan, and Mongoloid races [33].

The controls used in the present study represent 151 healthy individuals without T1DM and T2DM in the family history, and matched for ethnicity, geography and socioeconomic status and higher age compared to cases. Blood samples (3 ml) were collected into EDTA-coated vacutainers from both cases and controls with written informed consent. This research was approved by the Institutional Review Board of the Anthropological Survey of India as well as by the respective hospital’s ethical committee.

Genotyping
DNA was isolated according to the standard protocol [34]. Genotyping of the following HLA genes HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1 was performed using PCR followed by sequencing. The primers used in the present study to amplify different regions of aforementioned genes are documented in Table 1. A total volume of 10 μl was used for each PCR reaction which were carried out in an ABI Gene Amp PCR system 9700. The nucleotide

| Genotype | rs1047993 | TT | TC | CC | MAF (%) | HWE-p value | Dominant OR (p value) | Recessive OR (p value) | Allelic OR (p value) |
|----------|-----------|----|----|----|---------|-------------|----------------------|----------------------|---------------------|
| Cases    | 2 (1.45)  | 26 (18.84) | 110 (79.79) | 10.9 | 0.745   |             |                      |                      |                     |
| Control  | 2 (1.33)  | 18 (12.0)  | 130 (86.67) | 7.3  | 0.152   | 1.65 (0.114) | 1.09 (0.933)         | 1.54 (0.139)         |
| rs9272699| AA        | 15 (10.64) | 124 (87.94) | 6.7  | 0.068   |             |                      |                      |                     |
| Cases    | 0 (0)     | 15 (10.14) | 133 (89.86) | 5.1  | 0.516   | 1.22 (0.603) |                      | 1.35 (0.394)         |
| Control  | 0 (0)     | 17 (11.49) | 131 (88.51) | 5.7  | 0.459   | 1.35 (0.392) |                      | 1.60 (0.148)         |
| rs1048052| CC        | 17 (12.06) | 120 (85.11) | 8.9  | 0.003   |             |                      |                      |                     |
| Cases    | 4 (2.84)  | 17 (11.49) | 131 (88.51) | 5.7  | 0.459   | 1.35 (0.392) |                      | 1.60 (0.148)         |
| Control  | 0 (0)     | 17 (11.49) | 131 (88.51) | 5.7  | 0.459   | 1.35 (0.392) |                      | 1.60 (0.148)         |
| rs36219014| AA       | 17 (12.06) | 124 (87.94) | 6.0  | 0.446   |             |                      |                      |                     |
| Cases    | 0 (0)     | 17 (12.06) | 124 (87.94) | 6.0  | 0.446   |             |                      |                      |                     |
| Control  | 0 (0)     | 15 (10.27) | 131 (89.73) | 5.1  | 0.513   | 1.20 (0.631) |                      | 1.48 (0.642)         |
| rs707962 | GG        | 30 (20.69) | 115 (79.31) | 10.3 | 0.165   |             |                      |                      |                     |
| Cases    | 0 (0)     | 30 (20.69) | 115 (79.31) | 10.3 | 0.165   |             |                      |                      |                     |
| Control  | 0 (0)     | 19 (12.58) | 132 (87.42) | 6.3  | 0.409   | 1.81 (0.061) |                      | 1.72 (0.074)         |
| rs2308911| CC        | 15 (10.95) | 66 (48.18)  | 35   | 0.495   |             |                      |                      |                     |
| Cases    | 15 (10.95) | 66 (48.18) | 56 (40.88)  | 35   | 0.495   |             |                      |                      |                     |
| Control  | 14 (10.37)| 61 (45.19) | 60 (44.44)  | 33   | 0.795   | 1.16 (0.552) | 1.06 (0.877)         | 1.10 (0.610)         |
| rs2308912| AA        | 14 (10.0)  | 64 (45.71)  | 32.9 | 0.669   |             |                      |                      |                     |
| Cases    | 14 (10.0) | 64 (45.71) | 62 (44.29)  | 32.9 | 0.669   |             |                      |                      |                     |
| Control  | 12 (9.09) | 61 (46.21) | 59 (44.70)  | 32.2 | 0.502   | 1.02 (0.946) | 1.11 (0.799)         | 1.03 (0.870)         |
| rs707949 | CC        | 30 (20.69) | 115 (79.31) | 10.3 | 0.165   |             |                      |                      |                     |
| Cases    | 0 (0)     | 30 (20.69) | 115 (79.31) | 10.3 | 0.165   |             |                      |                      |                     |
| Control  | 0 (0)     | 20 (13.25) | 131 (86.75) | 6.6  | 0.384   | 1.71 (0.087) |                      | 1.63 (0.104)         |
| rs7990   | AA        | 14 (9.72)  | 130 (90.28) | 4.9  | 0.549   |             |                      |                      |                     |
| Cases    | 0 (0)     | 14 (9.72)  | 130 (90.28) | 4.9  | 0.549   |             |                      |                      |                     |
| Control  | 0 (0)     | 31 (20.53) | 120 (79.47) | 10.3 | 0.160   | 0.42 (0.009) |                      | 0.45 (0.013)         |
| rs707963 | GG        | 28 (19.31) | 117 (80.69) | 9.7  | 0.198   |             |                      |                      |                     |
| Cases    | 0 (0)     | 28 (19.31) | 117 (80.69) | 9.7  | 0.198   |             |                      |                      |                     |
| Control  | 0 (0)     | 18 (11.92) | 133 (88.08) | 6    | 0.436   | 1.77 (0.079) |                      | 1.69 (0.093)         |

MAF = Minor Allele Frequency, HWE = Hardy-Weinberg Equilibrium.
sequences of the PCR products were determined by direct sequencing using di-deoxy chain terminator cycle sequencing protocol through 3730 DNA Analyzer (BigDye V3.1, Applied Biosystems; Foster City, CA, USA). Sequencing was carried out with both the forward and the reverse directions. Problems in genotype assignment for certain samples on the DRB1 and the DQB1 loci that aroused because of the genotypic ambiguity were surmounted by following the guidelines of the American Society for Histocompatibility and Immunogenetics [35].

Statistical analysis
All nucleotide changes detected by using SeqScape software v 2.5 (Applied Biosystems) and with the wild gene using pair-wise BLAST [36]. Class II HLA genes are more polymorphic compared to other genes and thus the editing was much more complicated for Class II HLA. Allele and genotype frequencies of the SNP data compared between T1DM patients and healthy control groups. Statistical analysis for HWE was performed by Plink v 1.07 software [37]. Evaluation of the genotype or allele frequencies of cases and controls was carried out by calculating the odds ratios (OR) with 95% of confidence intervals (CI). A P value less than 0.05 were considered as statistically significant. Haplotype frequencies and linkage disequilibrium estimated by using Haplovie v 4.1, which measures D’ and r2 between each pair of SNPs and to define haploblocks [38].

Results and discussions
Exonic 331 SNPs in the Class II HLA- DP-DQ-DRB1, identified and tested for HWE by Plink software. Only ten SNPs found to be in equilibrium for control, only these 10 SNPs were used in further analysis. Of the 10 SNPs, eight were from HLA-DQA1 and the remaining were from HLA-DPA1 (rs2308911, rs2308912) (Table 2). Allele and genotype frequencies of all the SNPs for both cases and controls were presented in Table 2. Except for rs7990 (p = 0.009), there were no significant differences in genotype or allele frequencies of these SNPs between controls and T1DM cases (Table 2). Thus, after all prune and excluding from 331 SNPs, rs7990 SNPs of HLA-DQA1 shows a negative association with T1DM. The pairwise LD values (D’ and r2) among studied SNPs were provided in Figure 1 and in Table 3. Linkage
disequilibrium analysis had revealed strong LD and formed 3 haplotype blocks, suggested that haplotype
study might be useful. Haplotype-phenotype association using SNPs that were located in the LD blocks
in block 1 (rs1047993 and rs707949) showed association with T1DM (Table 3).

Conclusions
The complex nature of the HLA region on chromosome 6p21.31, with the high LD between genes, has made it
difficult to elucidate the exact nature of the risk of developing T1DM. The studies suggested that HLA Class II DRB1-DQB1 contribute
to T1DM susceptibility. Calculating for the influence of class II DR-DQ haplotype and genotype effects, a
role in T1DM has been shown of additional HLA Class II DPB1[16].

New studies by sequencing has yielded many SNPs that include thirteen SNPs from class III alleles, which
showed evidence of an effect on T1DM risk, although some of the SNPs are in tight LD with each other. The
strongest association within class III markers was with rs2395106 that maps 5’ to the NOTCH4 gene and the second
association was with rs707915 mapping to the MSH5 gene, in a block of six markers significantly associated with
T1DM after adjusting for LD with DR-DQ [39].

A widespread SNP analysis of the extended MHC in 237 families with Type 1A from the U.S. and 1,240 fam-
ilies from the T1DGC was conducted and showed an association with Type 1A diabetes (rs1233478, p = 1.6 x
10−23), in the UBD/MAS1L region, telomeric of the clas-
sic MHC [40]. Another study from T1DGC on HLA
markers showed 296 significant SNPs in a narrow ge-
nomic region, some of these markers are close to one
another and in strong LD. Therefore, although the SNPs
that stand for independent signals without LD, some
high-LD markers can produce correlated associations in
the study. However, high-LD and long haplotype blocks
will also deter fine mapping precisely. This study also
shows that SNPs with the smallest P values is from the
HLA-DR and -DQ region which confers the major ge-
etic risks for T1DM [41].

Most of these recent studies have used data produced by the T1DGC and therefore are from Caucasoid popu-
lation, and they conducted the most detailed investigation of the HLA complex in disease, characterizing over
3,000 SNPs, and independently tested all previously reported T1DM susceptibility genes [42,43].

In this study we used a sequencing SNP approach to characterize the polymorphisms in HLA genes. We
-carried out sequencing in 151 cases and 151 normal healthy participants, followed by statistical analysis of the
SNPs. Out of 331 exonic SNPs identified, only 10 is following HWE. Thus, after all pruning and excluding
from 331 SNPs, and rs7990 SNP of HLA-DQA1 showed significant protection from T1DM. Linkage disequilib-
rium analysis revealed 3 haplotype blocks and further haplotype-phenotype association analysis did show asso-
ciation between haplotypes in block 1 (rs1047993 and rs707949) and T1DM.

We have also done the study based on alleles and the
findings are similar like the DQA1*0103 allele is a novel allele with a significant association with the protection
from T1DM in Eastern Indian Bengali population from India [44]. To our knowledge, this study is the first of a
kind which has tried to check the HLA association with T1DM by SNPs analysis in India. As said before most of
these recent studies have used data formed by the T1DGC, and they use refined statistical methods to control
for the complexity of the HLA region because of the extended LD and polymorphic loci. Still the deviation of
some results suggests the difficulty of examine the inde-
pendent genetic contribution of genes in this region to
the risk of developing T1DM. It is most likely that more
SNPs with individual but smaller or rarer effects on dia-
abetes risk can be identified in this region. However, to
find these SNPs new approaches for analyzing genetic
association data are needed. In addition, large numbers of subjects may help to give more robust and confident
association with T1DM.

Competing interest
There are no conflicts of interest.

Authors’ contributions
OR carried out the molecular genetic studies, participated in the sequence alignment, did the statistical calculation and drafted the manuscript. BS corrected the manuscript. BLVKS conducted the Thesia calculation. VP suggested the project GS suggested the project SC provided with the
alignment, did the statistical calculation and drafted the manuscript. BS
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References
1. Atkinson M, Maclaren N: The pathogenesis of insulin dependent diabetes. N Engl J Med 1994, 331:1428–1436.
2. Imagawa A, Hanafusa T, Miyagawa J, Matsuzya Y: A novel subtype of type1 diabetes mellitus characterized by a rapid onset and an absence
of diabetes-related antibodies. N Engl J Med 2000, 342:301–307.
13. Weitkamp L: HLA and disease: predictions for HLA haplotype sharing in IMGT/HLA Database. 

10. Kelly M, Rayner ML, Mijovic CH, Barnett AH: Prevalence of type 1 diabetes mellitus in Karnal district, Haryana state, India. 

5. Ramachandran A, Snehalatha C, Abdul Khader OM, Joseph TA, Viswanathan M: Heterogeneity in the magnitude of the insulin gene effect on type 1 diabetes mellitus in patients from North Eastern India. 

14. Wolf E, Spencer KM, Cudworth AG: The genetic susceptibility to type 1 (insulin-dependent) diabetes: analysis of the type 1 diabetes genetics consortium. 

15. Florez J, Hirschhorn J, Altshuler D: The genetic susceptibility to type 1 diabetes: linkage and stratification analyses of 4,422 affected sib-pairs. 

22. Torn C, Gupta M, Zake NL, Sanjeevi CB, Landin-Olsson M: Association of MICA50/MICA51 and HLA-DR3-DQ2/DR4-DQ8 are independent genetic risk factors for latent autoimmune diabetes mellitus in adults. 

5. Ramachandran A, Snehalatha C, Krishnaswamy CV, Madras IDDM Registry Group-Madras: Incidence of IDDM in children in urban population in southern India. Madras IDDM Registry Group Madras, South India. 

11. Kelly M, Rayner ML, Mijovic CH, Barnett AH: Prevalence of type 1 diabetes mellitus in Karnal district, Haryana state, India. 

19. Kawakita E, Fujiyama M, and the association with type 1 diabetes mellitus in Bengali speaking patients of Eastern India. Diabetologia 2011, 40:99–106. 

20. Chattopadhyay S: The origin and development of Bengali languages. Calcutta: George and Unwin Ltd.; 1926. 

21. Singh K: Linguistic traits across language boundaries. Kolkata: Anthropological Survey of India; 1990. 

23. Majumdar D, Rao C: Race elements in Bengal: an attemptative study Indian statistical series-2. Calcutta: Asia Publishing House; 1960. 

24. Sambrook J, Russell DW: Molecular cloning: A laboratory manual. Cold Spring Harbor; New York: Cold Spring Harbor Laboratory Press; 2001. 

25. Noble J, Zilk A, Jin X, Adair BA, Moir H, Zhu Y, Shriver MD, Frühwald CM, Erlich HA, Fudenberg G: High-resolution sequence-based typing strategy for HLA-DQA1 using SSP-PCR and subsequent genotyping analysis with novel spreadsheet program. Tissue Antigen 2001, 58:308–314. 

27. Magzoub M, Stephens HA, Sachs JA, Biro PA, Cutbush S, Wu Z, Bottazzo GF: HLA-DP polymorphism in Sudanese controls and patients with insulin-dependent diabetes mellitus. Tissue Antigens 1992, 40:64–88. 

28. Yamagata K, Hanafusa T, Nakajima H, Sada M, Amerinma H, Tomita K, et al: HLA-DP and susceptibility to insulin-dependent diabetes mellitus in Japanese. Tissue Antigens 1991, 38:107–110. 

29. Cordovado S, Simone AE, Mueller PW: High-resolution sequence-based typing strategy for HLA-DQA1 using SSP-PCR and subsequent genotyping analysis with novel spreadsheet program. Tissue Antigen 2001, 58:308–314. 

30. Raha O, Sarkar BN, Bhakar LKVS, Veerapu P, Chowdhury S, Mukhopadhyay S, Biswas T, Rao V: Insulin (INS) promoter VNTR polymorphisms: interactions and the association with type 1 diabetes mellitus in Bengali speaking patients of Eastern India. Diabetologia 2011, 40:99–106. 

31. Chattopadhyay S: The origin and development of Bengali languages. Calcutta: George and Unwin Ltd.; 1926. 

32. Singh K: Linguistic traits across language boundaries. Kolkata: Anthropological Survey of India; 1990. 

33. Majumdar D, Rao C: Race elements in Bengal: an attemptative study Indian statistical series-2. Calcutta: Asia Publishing House; 1960. 

34. Sambrook J, Russell DW: Molecular cloning: A laboratory manual. Cold Spring Harbor; New York: Cold Spring Harbor Laboratory Press; 2001. 

35. Cano P, Klitz W, Mack SJ, Maires M, Marsh SCE, Noreen H, Reed EF, Szentpetri O, Setzler D, Rehder H, Smith A, Fernández-víllosa M: Common and well-documented HLA alleles: report of the ad-hoc committee of the American society for histocompatibility and immunogenetics. Hum Immunol 2007, 68:392–417. 

36. Tatsuoka T, Wadden T: BLAST 2 sequences a new tool for comparing protein and nucleotide sequences. FEBS Microbiol Lett 1999, 174:247–250. 

37. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PJ, Daly MJ, Sham PC: PLINK: a toolset for whole-genome association and population-based linkage analysis. Am J Hum Genet 2007, 81:565–574. 

38. Barrett J, Fry B, Maller J, Daly MJ: Haplview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005, 21:263–265. 

39. Valdes A, Thomson G: Type 1 Diabetes Genetics Consortium: Several loci in the HLA class II region are associated with T1D risk after adjusting for DRB1-DQB1. Diabetes Obes Metab 2009, Suppl 1:46–52. 

40. Ayyasamy P, Neale B, Todd-Brown K, Thomas L, Feneira MAR, Bender D, Maller J, Sklar P, de Bakker PJ, Daly MJ, Sham PC: PLINK: a toolset for whole-genome association and population-based linkage analysis. Am J Hum Genet 2007, 81:565–574. 

41. Barrett J, Fry B, Maller J, Daly MJ: Haplview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005, 21:263–265. 

42. Valdes A, Thomson G: Type 1 Diabetes Genetics Consortium: Several loci in the HLA class II region are associated with T1D risk after adjusting for DRB1-DQB1. Diabetes Obes Metab 2009, Suppl 1:46–52. 

43. Ayyasamy P, Neale B, Todd-Brown K, Thomas L, Feneira MAR, Bender D, Maller J, Sklar P, de Bakker PJ, Daly MJ, Sham PC: PLINK: a toolset for whole-genome association and population-based linkage analysis. Am J Hum Genet 2007, 81:565–574. 

44. He C, Hamon S, Li D, Basral-Rodriguez SJO, Diabetes Genetics Consortium: MHC fine mapping of human type 1 diabetes using the T1DGC data. Diabetes Obes Metab 2009, 11:53–59. 

45. Barrett JC, Clayton DG, Concannon P, Alkbuurk B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, et al: Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009, 41:703–707. 

46. Morahan G, Mehta M, James I, Chen WM, Alkbuurk B, Erlich HA, Hilner JE, Julier C, Nerup J, Nierras C, et al: Tests for genetic interactions in type 1 diabetes: linkage and stratification analyses of 4,422 affected sib-pairs. Diabetes 2011, 60:1030–1040. 

47. Raha O, Sarkar BN, Veerapu P, Buddhakar G, Raychaudhuri P, Mukhopadhyay S, Rao V: Role of HLA class II loci polymorphism in the manifestation of type 1 diabetes in a Bengali Indian patient population. Genet Test Mol Biomarkers 2013, 17:52–61.