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# 14th International HLA and Immunogenetics Workshop: Report on the HLA component of type 1 diabetes

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## Abstract

The type 1 diabetes (T1D) component of the 13th International Histocompatibility Workshop (IHW) obtained microsatellite (msat) and human leukocyte antigen (HLA)-DR/DQ data on case/control and family samples through an international collaboration. The aim was to detect the effects of susceptibility loci on the HLA complex independent of the primary determinants in the class II region (HLA-DR/DQ). As part of the activity of the 14th International HLA and Immunogenetics Workshop (14th IHIWS), a T1D workshop was held to present analyses of the 13th IHW data and to discuss the current status of knowledge about the genetics of T1D. These data are now available online through dbMHC, a web-based resource established by the National Center for Biotechnology. Continuing work since the 13th IHW has resulted in published work showing heterogeneity of DR3 haplotypes in data sets from the 13th IHW and Human Biological Data Interchange (HBDI). In addition, we identified markers that define DRB1*1501 DQB1*0602 haplotypes conferring reduced protection from diabetes in a Swedish 13th IHW data set. Further analyses of the 13th IHW data set not only showed some significant results but also demonstrated extensive heterogeneity reminiscent of non-HLA genes. The haplotype analysis in HBDI families identified two msats with significant effects on susceptibility and statistically significant age of onset effects at class III markers that are not because of linkage disequilibrium, with class I alleles known to affect age of onset. The above studies underscore the importance of refining our understanding of susceptibility associated with genes in the HLA complex.

## Introduction

### Historical context and overview

The activities of the type 1 diabetes (T1D) component of the 13th International Histocompatibility Workshop (IHW) have spanned through the 13th and 14th International HLA Workshops. Both scientific and technological progress

*These authors contributed equally to this work.
†See Appendix for full listing of 13th IHWS Type 1 Diabetes Component participating investigators.

demanded a different approach for the planning and execution of this collaborative effort. Earlier workshops involved the distribution of standardized reagents to participating centers, the typing of available DNA samples, and the reporting of the data for joint analysis to be presented at the workshop meeting (1). The T1D component of the 13th IHW built on these efforts and involved a large number of centers, including many additional ones that had not participated in earlier workshops. This effort was designed to test specific hypotheses, supported by preliminary data reported in Lie et al. (2) and involved a combined approach for studying human leukocyte antigen (HLA) alleles by DNA-based typing as well as by microsatellite (msat) typing. This was the first time that msat typing was included in a workshop diabetes study. Logistical considerations imposed the need to identify a core laboratory for msat typing, the Center National de Genotypage, as most of the HLA participating centers were not equipped for msat typing. An additional core laboratory was identified to perform high-resolution HLA typing on selected samples. Research grant funds were available to support the typing of samples obtained from existing DNA collections. This effort resulted in the collection of large, multiethnic case–control and family cohorts, with the collection of a large number of families with a parent homozygous at the HLA class II DR/DQ loci. This was an important innovation in the study design and underscores the importance of collaboration as a parent homozygous for DR-DQ is identified in only 10%–20% of the families. This approach resulted in the establishment of a large, international collaboration, the typing of multiple msat markers in more than 10,000 samples, the collection of available phenotypic and demographic data, and the establishment of a new, freely accessible web-based database, dbMHC, developed in collaboration with National Center for Biotechnology (NCBI).

### Scientific background and rationale

The HLA class II genes DRB1-DQB1 represent the major genetic susceptibility component to T1D. However, there is evidence that several other genes in the HLA region also contribute to T1D (3). The effect of other class II (DPB1) (4, 5) and class I genes (6, 7) has been shown, as well as DR-DQ haplotype-specific effects were detected using class I loci as markers (8, 9) or msat marker loci (2, 10–12). The aim of the 13th IHW T1D study was to consolidate the resources of the HLA community to assemble a large and ethnically diverse data set designed to detect the role of additional genes in the HLA region involved in disease in the context of the classical HLA-DR/DQ genes. In particular, the major objective was to investigate the effects of additional T1D susceptibility genes in the HLA complex, controlling for the strong influence of common high-risk class II haplotypes (i.e. DRB1*0301-DQB1*0201, DRB1*0401-DQB1*0302, DRB1*0404-DQB1*0302).

This report will review the analyses performed on the 13th IHW data, the development of dbMHC, and present further analyses based on the application of the haplotype method, with special emphasis on hypothesis-driven approaches. Detailed results on the haplotype analyses and studies of age of onset effects in the HBDI families are also presented, as these families were also included in the 13th IHW study. Finally, this report will summarize the workshop activities and discussions that took place in Melbourne during the 14th IHIWS.

## Subjects, genetic markers, and methods

### 13th IHW T1D data sets

Thirty-three laboratories participated in this study, contributing a total of 44 independent data sets. Families from 19 populations included those characterized by having a parent homozygous at the DRB1-DQB1 loci as well as randomly ascertained samples, for a total of 1117 families. Case–control studies were also conducted for the T1D component. Overall, a total of 3178 T1D cases and 2275 controls were available from 25 populations. Some were randomly selected, some were selected for specific DRB1-DQB1 genotypes, and some were specifically 1 : 1 matched for DRB1-DQB1. All data sets were subjected to high-resolution HLA-DRB1 and -DQB1 typing for the HLA-DR4 haplotypes. DNA samples were genotyped at the Centre National de Genotypage as previously reported (13). Data on eight msat loci were available for analysis: D6S2239, D6S2223, D6S2222, D6S265, MIB2, TNFd, D6S273, and D6S291.

### 13th IHW T1D haplotype analyses

The following data sets were analyzed: FRAFAJ (case–control, France), GBRGIL (family, UK), FINETW (family, Finland), FINTIL (family, Finland), DNKNER (family, Denmark), ITACUR (case-control, Italy), ESPANO [family, Spain (Basque)]. For the case–control data, haplotypes (DR-DQ plus msat) were estimated using PyPop (http://www.pypop.org) (14, 15). Because the EM algorithm for haplotype estimation assumes Hardy–Weinberg proportions (HWP), those 13th IHW samples with large departures from HWP were excluded from the analyses presented here. For the family data, the AFBAC method of Thomson (16, 17)] was used (nontransmitted parental alleles and haplotypes form the control population). Transmitted and nontransmitted alleles were determined, and haplotypes were constructed using this information. If DR-DQ haplotype frequencies for case–control or transmitted-AFBAC groups totaled less than 20, the haplotype (msat

frequencies on specific DR-DQ haplotypes) analysis was not performed. Those individual msat alleles that had five or fewer counts were also deleted. Goodness of fit chi-squares are reported, as well as the standardized chi-square is described by Valdes and Thomson (18). An empirical confidence interval (CI) for the standardized chi-square was obtained using 1000 bootstrap samples from the DR-DQ-msat distribution; the standardized chi-square was determined, and an empirical CI taken from the distribution of the standardized chi-squares.

### HBDI family data

The HBDI has established a large collection of Caucasian families from the United States, with multiple members affected by T1D (19). Family data on HLA classical and msat loci were kindly provided by Patrick Concannon and Janelle Noble. DNA samples from 208 Caucasian, multiplex families were obtained from the HBDI collection. Only two affected sibs from each family were used. Analyses were carried out on high-resolution HLA DR-DQ data, age of onset information, and 10 msat markers in the HLA region: D6S2239*, D6S276, D6S2672, TNFa, D6S273*, N325, D6S2669, DRA, DQCARII, and D6S2662 (the two markers indicated with * were also typed in the 13th IHW data set). The most likely haplotype phase at those markers plus DRB1-DQB1 was assigned using SIMWALK 2.83 (©1995–2002 E. Sobel). Haplotype analysis of msats stratified by the highly predisposing DR3 and DR4 haplotypes was carried out using patient and AFBACs as 'controls' data (16, 17) and using the transmission disequilibrium test (TDT) (20). The overall identity by descent (IBD) distribution for the genetic region studied was IBD(0) = 5.3%, (IBD)(1) = 40.3%, and IBD(2) = 54.4%. Under the null hypothesis that within the HLA region, no other loci modify the effect on T1D susceptibility of these haplotypes, we expect the proportion transmitted to offspring affected with T1D from heterozygote parents to be the same for all of the msat alleles in the predisposing DR3 and DR4 haplotypes. Deviations from such random expectation were assessed using a chi-squared test where the total sum in the contingency table was greater than 200 or using a Fisher's exact test otherwise.

### Age of onset effects

Age of onset effects were evaluated on all patient samples as well as separately among patients carrying the high-risk DR3/DR4 genotype and those not carrying it. Patients were classified as DR3/DR4 if they carried both a DRB1*0301, DQB1*0201 haplotype and a DRB1*0401/2/4/5, DQB1*0302 or DRB1*0405, DQB1*0201 haplotype. Patients carrying a DR3 haplotype but other DRB1*04 subtypes or not carrying a DQB1*0302 or DQB1*0201 allele were not classified as DR3/DR4. For those alleles that showed a significant effect on age at onset, we explored whether this effect could be explained by linkage disequilibrium (LD), with specific class I alleles known to affect age of onset in this cohort. Analyses of variance (ANOVAS) were carried out using S-PLUS 6.1 (Insightful Corp, Seattle, WA), with the natural logarithm of age of onset as the outcome variable and the presence of at least one copy of an allele (1) or zero copies of an allele (0) as the independent variable. When both the patients with DR3/DR4 and without DR3/DR4 haplotypes were analyzed together ('all samples'), the DR3/DR4 status of the patient (0 or 1) was included as a covariate in the ANOVA.

### Significance levels

For all analyses, uncorrected *P* values are given. Many of the results reported would no longer be significant with correction, so caution in any interpretation is needed. We have taken this approach because replication in other data sets is the best approach to validate true effects.

## Results

### Online database – dbMHC

The data from this collaborative effort are now available online via the NCBI's dbMHC (http://www.ncbi.nlm.nih.gov/mhc). The database contains data from 27 laboratories worldwide that provided samples from populations with diverse racial/ethnic backgrounds. Data are available on 2957 patients and 1810 controls from 23 different case–control study data sets, and six data sets consisting of 897 families, of which 433 have one parent homozygous at the HLA-DR/DQ. In total, more than 8000 samples were typed, resulting in 81,266 genotypes of HLA class II genes, and eight msats spanning the extended major histocompatibility complex (MHC) region. Custom subsets of these data can be retrieved based on user specified criteria. Users may filter the data set to include samples from specific populations or that have specific observed alleles (allelic groups) at HLA-DRB1, DQA1, and DQB1 and the eight msat loci. Cumulative frequency or individual sample display is selected by the user. Family genotype data can also be viewed in the dbMHC pedigree view. Download of the entire data, or subset, is available in tab delimited, comma separated text, or XML. Searchable data fields include demographic information, ethnic origin, submitting laboratory, sex, clinical information, affection status, age of onset, family history, and autoantibody titers. This resource will continue to grow with the addition of new data generated by consortia or by individual investigators.

### Analysis of the 13th IHW data sets

In our report of initial analyses of the 13th IHW data (13), statistical methods for T1D families were based on the

homozygous parent TDT (HPTDT) and matched DRB1-DQB1 homozygous cases and controls from both family and case–control data. Those preliminary analyses suggested that the most centromeric (D6S291) and telomeric (D6S2239) loci are in LD with genes that may increase T1D risk. There was evidence that candidate genes in the class III – I region (i.e. TNFd, MIB, and D6S265), which potentially reflect the HLA-A and -B loci, may also be involved. There was also suggestive evidence confirming a potential locus near D6S2223/D6S2222 on DRB1*0301-DQB1*0201 haplotypes. While some significant and consistent results were found overall in the analyses, the results showed extensive heterogeneity between data sets. Importantly, the results were reminiscent of non-HLA genes in complex diseases.

Additional analyses, which were recently published (21) and presented at the 14th IHIWS, involved the HLA DQB1*0602 allele. This allele confers strong dominant protection against T1D although protection is not absolute (22). Eight patients in the 13th IHW Swedish (USALER) data set carried the DRB1*1501 DQB1*0602 haplotype. Despite this small number, analysis of the eight msat loci typed on the 13th IHW data found an allele at D6S265 associated with weaker protection (21). Allele 15 at marker D6S265 (109 kb centromeric of HLA-A) was increased among patients with T1D carrying DRB1*15-DQB1*0602. The DRB1*15 DQB1*0602 haplotypes carrying D6S265*15 have an odds ratio 10-fold higher than those carrying other alleles. Thus, typing for allele D6S265*15 can identify a less protective DQB1*0602 haplotype, allowing a more accurate prediction of T1D risk. Study on additional patients and controls with respect to this effect are in progress.

Table 1 shows the results of further DR-DQ msat haplotype analyses. While a number of markers showed significant effects (uncorrected *P* values), there was no consistency across markers or populations or DR-DQ haplotypes. This also applies to the few markers with significance levels <0.01. Overall, these analyses confirm heterogeneity across populations and highlight the importance of sample size. Using the bootstrap CIs, no significant effects were seen.

**Table 1** Haplotype method analyses of the 13th IHW data

| DR/DQ | Microsatellite | Laboratory | Case–control or family | Goodness of fit chi-square | Unadjusted *P* value | Standardized chi-square | Bootstrap 95% CI |
|---|---|---|---|---|---|---|---|
| 0301/0201 | D6s291 | FRAFAJ | Case–control | 12.708 | 0.026 | 2.438 | (0.639, 7.402) |
| | D6s273 | ITACUR | Case–control | 10.133 | 0.038 | 2.168 | (−0.024, 8.095) |
| | TNFd | ITACUR | Case–control | 8.399 | 0.015 | 3.199 | (0.254, 10.611) |
| | MIB | ITACUR | Case–control | 19.034 | 0.002 | 4.438 | (1.822, 9.397) |
| | D6s265 | | | | | | |
| | D6s2222 | FRAFAJ | Case–control | 10.913 | 0.004 | 4.457 | (0.810, 11.591) |
| | | GBRGIL | Family | 6.187 | 0.045 | 2.093 | (−0.607, 8.559) |
| | D6s2223 | FRAFAJ | Case–control | 6.634 | 0.036 | 2.317 | (−0.673, 9.736) |
| | | GBRGIL | Family | 4.545 | 0.033 | 2.507 | (0.275, 5.941) |
| | D6s2239 | DNKNER | Family | 6.411 | 0.041 | 2.205 | (−0.330, 6.688) |
| 0401/0302 | D6s291 | FRAFAJ | Case–control | 14.728 | 0.001 | 6.364 | (3.097, 9.9) |
| | D6s273 | FRAFAJ | Case–control | 7.504 | 0.023 | 2.752 | (−0.092, 8.335) |
| | TNFd | FRAFAJ[a] | Case–control | 4.218 | 0.040 | 2.276 | (−0.61, 10.634) |
| | | FINETW | Family | 3.629 | 0.057 | 1.869 | (−0.668, 8.435) |
| | MIB | FRAFAJ | Case–control | 11.661 | 0.003 | 4.83 | (0.634, 11.846) |
| | D6s265 | | | | | | |
| | D6s2222 | FRAFAJ | Case–control | 4.635 | 0.031 | 2.571 | (−0.602, 9.083) |
| | D6s2223 | | | | | | |
| | D6s2239 | FRAFAJ | Case–control | 9.45 | 0.009 | 3.725 | (0.211, 10.851) |
| | | GBRGIL | Family | 7.183 | 0.028 | 2.591 | (−0.701, 11.347) |
| | | DNKNER | Family | 8.199 | 0.017 | 3.099 | (0.024, 9.379) |
| | | FINETW and FINTIL | Family | 5.85 | 0.054 | 1.925 | (−0.253, 7.367) |
| 0404/0302 | D6s291 | | | | | | |
| | D6s273 | | | | | | |
| | TNFd | | | | | | |
| | MIB | | | | | | |
| | D6s265 | | | | | | |
| | D6s2222 | FRAFAJ | Case–control | 11.435 | 0.001 | 7.379 | (3.393, 13.435) |
| | D6s2223 | | | | | | |
| | D6s2239 | FINETW and FINTIL | Family | 3.638 | 0.056 | 1.865 | (−0.684, 9.049) |

CI, confidence interval; IHW, International Histocompatibility Workshop.

[a] TNFd is not in Hardy–Weinberg equilibrium for controls for FRAFAJ ($P < 0.05$).

## HBDI analyses

### Haplotype method results

In analysis of HBDI families using the haplotype method, testing for heterogeneity of msat frequencies on specific DR-DQ haplotypes (8, 18), 2/11 msats showed significant effects: D6S276 on DRB1*0401 DQB1*0302 haplotypes (Table 2) and D6S273 (a 13th IHW marker) on DR3 haplotypes (Table 3). The DR3 results were further investigated in both the HBDI data (23) and the 13th IHW data sets (Dorman, University of Pittsburgh, Pittsburgh, unpublished data): we found that HLA class III marks an extended DR3-B18 haplotype associated with increased susceptibility to T1D in both samples, in agreement with previous results (12). Further results for the HBDI data indicated that multiple alleles of D6S273 mark a susceptibility locus whose effect we were able to detect only among DR3 haplotypes but not limited to DR3-B18 haplotypes.

### Age of onset analysis

There is evidence from twin studies that T1D with younger age of onset (<10 years) has a stronger genetic component than if disease in the index twin was diagnosed later in life (e.g. >24 years) (24, 25). Moreover, a young age of onset might be indicative that the process leading to β-cell destruction and T1D has occurred within a relatively short interval. HLA class II and I differences related to age at onset have been reported both in the HBDI (7, 26) and in other cohorts. Genetic variation within the HLA region not directly at the class II DRB1-DQB1 or class I A, B or C loci influencing age of onset of T1D can help us identify additional loci within the HLA that modify disease susceptibility and progression. Our study of the HBDI data, using msats and HLA class I data in conjunction with HLA DR-DQ, shows age of onset effects at class III msat markers, which are independent of known class I effects. Among individuals carrying the DR3/DR4 genotypes, alleles in the D6S2672 locus were strongly associated with younger age at onset (Table 4). However, these alleles are in strong LD with HLA class I alleles such as B*3906, C*0702, and A*2401, which have been previously shown in these same samples to be associated with younger age at onset (6, 7). The role of class I loci in the CD8 T-cell cytotoxic destruction of β cells makes them more credible candidates for a role in age at the onset of T1D than anonymous markers. In addition, statistically significant ($P < 0.05$) effects on age of onset among individuals carrying DR3/DR4 were seen at D6S2669 and D6S2662 (Table 5), while in individuals not carrying DR3/DR4significant effects were seen at alleles N325* 4 and DQCARII (Table 6). These markers are located within the MHC class II and III, and their effects on age of onset cannot be explained by LD with class I alleles. Allele D6S2669 *1 was strongly associated with significantly older age of onset, but its effect is likely to be because of LD with specific class I alleles (Table 7).

## Additional analyses presented at the 14th IHIWS

Besides the analysis reported above, additional results discussed during the workshop included additional evidence that the D6S2223 marker in the extended class I region modifies risk on DR3 haplotypes and that additional risk loci may be located telomeric of D6S2223, as presented by Benedicte Lie. She also reviewed recently published data on the LD patterns, haplotype blocks, and tag selection throughout the entire extended MHC for DR3 and DR4 haplotypes (27). Significant differences in LD were observed, demonstrating that the architecture of the underlying ancestral haplotypes must be considered when constructing LD maps and may be critical for mapping of disease susceptibility genes.

Recent analysis on the HBDI families discussed by Henry Erlich suggested further influence from class I alleles; the diabetes-protective allele B*4403 was associated with older

**Table 2** D6S276 allele frequency distribution among transmitted and nontransmitted DRB1*0401 DQB1*0302 haplotypes in the HBDI data

| DR-DQ | D6S276 | Frequency of transmission | Frequency of nontransmission | % transmitted | *P* value |
|---|---|---|---|---|---|
| 0401-0302 | *1 | 0.067 | 0.195 | 91.3 | 0.031 |
| | *5 | 0.033 | 0.112 | 92.3 | 0.081 |
| | *6 | 0.000 | 0.009 | 100.0 | n.s |
| | *7 | 0.133 | 0.126 | 77.1 | n.s |
| | *9 | 0.000 | 0.014 | 100.0 | n.s |
| | *10 | 0.100 | 0.037 | 57.1 | 0.057 |
| | *12 | 0.133 | 0.047 | 55.6 | 0.020 |
| | *13 | 0.467 | 0.321 | 71.1 | n.s |
| | *14 | 0.067 | 0.130 | 87.5 | n.s |
| | *17 | 0.000 | 0.009 | 100.0 | n.s |
| Total | | 60 | 215 | 78.2 | |

HBDI, Human Biological Data Interchange, n.s., not significant.

**Table 3** D6S273 allele frequency distribution among transmitted and nontransmitted DR3 in the HBDI data

| DR-DQ | D6S273 | Frequency of transmission | Frequency of nontransmission | % transmitted | *P* value |
|---|---|---|---|---|---|
| 0301-02 | *1 | 0.026 | 0.009 | 85.7 | n.s. |
| | *2 | 0.187 | 0.096 | 80.0 | 0.042 |
| | *3 | 0.013 | 0.017 | 60.0 | n.s. |
| | *4 | 0.051 | 0.061 | 63.2 | n.s. |
| | *5 | 0.085 | 0.017 | 90.9 | 0.018 |
| | *6 | 0.017 | 0.017 | 66.7 | n.s. |
| | *7 | 0.579 | 0.765 | 60.7 | 0.041 |
| | *8 | 0.043 | 0.017 | 83.3 | n.s. |
| Total | | 251 | 125 | 67.1 | |

HBDI, Human Biological Data Interchange; n.s., not significant.

age at onset, while the predisposing alleles C*0702 and B*3906 were associated with younger age at onset (7). There was also discussion about the importance of cross-ethnic studies; if large enough, this approach can enable validating alleles as true susceptibility factors, including DR9 and DRB1*0401, according to data presented by Henry Erlich and Narinder Mehra. Studies in the Australian population using 15 SNPs helped define the TNF block as 17 haplotypes that account for 90% of the Australian population, which were presented by Patricia Price (28). The definition of this haplotype can be used as a mapping tool for T1D, other diseases and anthropological studies. These studies also provide additional evidence for a susceptibility gene in the central region of the HLA complex in linkage with DRB1*0401 (28).

## Summary of 14th IHIWS discussions

The role of HLA genes in T1D was debated not only at the genetic level but also in terms of molecular mechanisms, cellular interactions, and clinical applications. From the functional point of view, there is growing evidence that the class II and I antigens associated with increased T1D risk act as restricting elements for T-cell responses against a variety of islet autoantigens [reviewed by Lieberman and DiLorenzo (29)]. Given that the primary genetic association is with the class II region, it is not surprising that the vast majority of T-cell epitopes identified so far are restricted by class II alleles, especially DR4 and DQA1*0301/DQB1*0302 (DQ8). Fewer class I-restricted T-cell epitopes have been identified so far. The technology to identify T-cell epitopes has progressed steadily over the past few years, but a systematic and comprehensive survey has not been performed. Thus, the difference may not reflect the actual prevalence of CD4 and CD8 responses during disease progression.

It is also plausible that class II and I antigen-presenting molecules may play a role in the selection of the T-cell repertoire. The recent discovery that self-molecules with a tissue-restricted expression, such as insulin, are ectopically expressed in the thymus and peripheral lymphoid tissue by thymic epithelial cells and a subset of dendritic cells, raising the possibility that genetic polymorphisms involving antigen-presenting molecules may influence presentation to developing T cells and in turn the efficiency of thymic selection processes, with obvious implications for self-tolerance and the risk of autoimmunity [reviewed by Pugliese (30)]. A limitation of our current knowledge about the presentation of peptide antigens by specific HLA molecules is that most studies are based on the analysis of cell lines although this has allowed for much progress in the definition of antigens that are presented by diabetes-predisposing molecules (31). It would be important to define how HLA molecules affect the presentation of molecules that are naturally expressed and presented by antigen-presenting cells. Several questions about the role of HLA molecules in relation to self-antigen presentation and tolerance remain unanswered, including whether there are different outcomes depending on the antigen-presenting molecule, allele-wise and locus-wise, and whether disease-predisposing antigen-presenting molecules differ from

**Table 4** D6S2672 alleles associated with age at onset of T1D on DR3/4 patients in the HBDI ($n = 169$)

| Locus | Allele | Number of carriers | Carrier frequency (%) | *P* | Noncarrier age at onset (years) | SE | Carrier age at onset (years) | SE |
|---|---|---|---|---|---|---|---|---|
| D6S2672 | 2 | 13 | 7.69 | <0.007 | 11.00 | 0.60 | 7.08 | 2.06 |
| | 4 | 27 | 15.98 | <0.025 | 11.25 | 0.63 | 7.82 | 1.42 |

HBDI, Human Biological Data Interchange; T1D, type 1 diabetes.

**Table 5** Markers and alleles in the HBDI showing an effect on age at onset of T1D on all patients ($n = 436$)

| Locus | Allele | Number of carriers | Carrier frequency (%) | *P* | *P*< incl. DR3/4 | Noncarrier age at onset (years) | SE | Carrier age at onset (years) | SE |
|---|---|---|---|---|---|---|---|---|---|
| D6S2669 | 1 | 6 | 1.38 | <0.022 | 0.032 | 11.92 | 0.40 | 20.00 | 3.21 |
| D6S2662 | 16 | 29 | 6.65 | <0.044 | 0.0876 | 11.54 | 0.39 | 14.90 | 1.45 |

HBDI, Human Biological Data Interchange; T1D, type 1 diabetes.

disease protecting ones in their ability to negatively select autoreactive T cells or stimulate regulatory T cells.

In terms of disease risk prediction and related clinical applications, typing for selected HLA alleles can identify high-risk individuals. The earliest identification of at-risk subjects is critical to the success of primary prevention strategies. In the DAISY study, which screens newborns for genetic markers of T1D, ~2% of the newborns in Colorado have the high-risk genotype and comprise almost 50% of those children who will develop diabetes by age 5 years (32). In the population-based Bart's Oxford study (UK), Lambert et al. (33) defined the absolute T1D risk defined by HLA class II genotypes. The highest risk genotype had 22.6% sensitivity and 24.7% specificity overall. If the six highest risk genotypes were used, sensitivity rose to 48.4% (0–4 year old), 32.7% (5–9 year old), and 28.96% (10–14 year old). The absolute risk for the highest risk genotype was 17/1000 and 51/1000 by age 5 and 15 years, respectively, with a cumulative incidence in the population of 0.6/1000 and 3/1000 by age 5 and 15 years. Such a risk is close to that of a first-degree relative but applies to a much larger proportion because family history is present in only ~10% of diagnosed children. Thus, HLA can identify at-risk subjects, but the logistics of HLA genetic screening for primary prevention in the general population remains a major obstacle to its implementation. There is also evidence that a family history of multiple affected family members can be used as an additional risk factor and allows for higher sensitivity in the identification of at-risk individuals for primary prevention (34). While a number of prevention trials have been carried out during the past several years, HLA typing has not been a major entry criteria or a criteria for risk assessment (35). HLA information has been analyzed retrospectively in most studies, but so far, it has been collected only of study participants and not in family members. At the workshop, Pam Fain presented data supporting the importance of taking into account HLA alleles and HLA sharing as risk factors in the context of prevention trials, as this may allow to identify the subjects with higher risk. Presenting data from the family studies ongoing at the University of Colorado, she reported that DR3/four siblings sharing two haplotypes have 65% risk of T1D by age 12 *vs* 20% of those sharing one or zero haplotypes. Thus, it is becoming increasingly evident that HLA typing can and should be applied more extensively in the clinical research arena. As noted, HLA typing is a screening tool for primary prevention. Typing for diabetes-protective alleles helps excluding those at low risk, but studies in those with autoimmunity may help studying natural protective mechanisms. HLA genotypes, sharing and positive multiplex family history are additional risk factors that can be integrated into risk assessment in natural history studies and prevention studies, thus helping identifying high-risk subjects. The interpretation and prediction of responses to treatment and of mechanistic studies, especially T-cell studies, is heavily dependent on knowing the HLA status. As recently shown in mouse studies, the administration of a proinsulin peptide may elicit a population of regulatory CD4 T cells, but binding of the same peptide to class I molecules stimulated a cytotoxic CD8 T-cell response (36). This study showed the importance of characterizing binding of potentially therapeutic peptides to both class II and class I molecules to identify those peptides that, depending on HLA genotypes at the main class II and class I loci, are more likely to have therapeutic effects and less likely to promote disease activity. Thus, HLA typing can help in understanding the interactions of certain HLA molecules with antigenic peptides in the context of antigen-based therapy and predict unwanted outcomes (37).

**Table 6** Markers and alleles in the HBDI showing an association with age at onset of T1D on patients not carrying DR3/4 ($n = 267$). (Only significant associations not in LD with class I markers also associated with age at onset are shown)

| Locus | Allele | Number of carriers | Carrier frequency (%) | *P* | Noncarrier age at onset (years) | SE | Carrier age at onset (years) | SE |
|---|---|---|---|---|---|---|---|---|
| N325 | 4 | 15 | 5.62 | <0.026 | 11.94 | 0.51 | 16.33 | 2.01 |
| DQCARII | 13 | 22 | 8.24 | <0.010 | 11.91 | 0.51 | 15.77 | 1.67 |
| | 17 | 18 | 6.74 | <0.047 | 12.00 | 0.51 | 15.39 | 1.85 |

HBDI, Human Biological Data Interchange; LD, linkage disequilibrium; T1D, type 1 diabetes.

**Table 7** Per cent of haplotypes carrying a specific marker allele also carrying class I alleles previously reported to influence age of onset in the HBDI cohort

| Locus | Allele | Effect on age of onset | Haplotypes with class I alleles |
|---|---|---|---|
| N325 | 4 | Older | 5% with old age of onset class I alleles |
| DQCARII | 13 | Older | 0% with old age of onset class I alleles |
| DQCARII | 17 | Older | 41% with old age of onset class I alleles |
| D6S2672 | 2 | Younger | 28%with young age of onset class I alleles |
| D6S2672 | 4 | Younger | 82% with young age of onset class I alleles |
| D6S2669 | 1 | Older | 67% with old age of onset class I alleles |
| D6S2662 | 16 | Older (n.s.) | 0% with old age of onset class I alleles |

HBDI, Human Biological Data Interchange; n.s., not significant.

Another important area of discussion centered on the study of non-HLA genes. When analyzed in the context of overall disease susceptibility, it is clear that the HLA region provides by far the strongest contribution (38). However, there is also evidence for several additional susceptibility loci. Many of these loci show modest linkage, and the linkage is often not confirmed in all genome scans. Sample size and composition, genetic heterogeneity and analytical methods underlie much of the variability observed in these studies. A coordinated effort to investigate the genetics of the disease, the Type 1 Diabetes Genetics Consortium (T1DGC) (www.t1dgc.org), has been launched and involves the study of patients and their families from around the world. In 2005, the consortium published its first report (38), which was presented at the workshop by Steve Rich. This was a combined linkage analysis of four data sets, three previously published genome scans, and a new data set of 254 families. This analysis included of 1435 families with 1636 affected sibling pairs, representing one of the largest linkage studies ever performed for any common disease and involving families from the United States, UK, and Scandinavia. Given the average map information content (67%, >400 polymorphic msat markers in each scan), this data set had ~95% power to detect a locus with $\lambda_s \geq 1.3$ and $P = 10^{-4}$. With this analytical power, more than 80% of the genome was found not to harbor susceptibility genes of modest effect that could be detected by linkage. The study confirmed linkage with HLA (nominal $P = 2.0 \times 10^{-52}$). Moreover, nine non-HLA-linked regions showed some evidence of linkage (nominal $P < 0.01$), including three at genome (or near)-wide significance ($P < 0.05$): 2q31-q33, 10p14-q11, and 16q22-q24. In addition, after taking into account the linkage at the 6p21 (HLA) region, there was evidence of linkage with the 6q21 region (*IDDM15*). Further, data discussed by Pat Concannon showed that the HLA higher risk genotype (DR4-DQB1*0302/DR3-DQB1*0201) confers an odds ratio of about 30, compared with only 1.9–1.2 for non-HLA loci such as the insulin gene, PTPN22 and CTLA4.

At the end of the workshop activities, a consensus was reached on recommendations for future progress. Based on the critical contribution to diabetes risk conferred by the HLA region, some of which remains unexplained, it was concluded that further studies aiming at refining the knowledge of HLA-associated susceptibility are critical to better understand better disease pathogenesis and improve prediction for clinical applications. It was recognized that further progress is likely to come from continued collaboration and international consortia and that it would be important to establish a renewable core of investigators to maintain continuity and build on the progress made so far by the HLA community, to develop interactions with the T1DGC, and to increase interactions with participating investigators. There exist an important resource of DNA samples that has been studied by this and earlier workshops, which would be important to continue to study. With the advent of whole genome amplification, it should be possible to regenerate these DNA samples and to continue their characterization. This was suggested as a potential area of interaction with the T1DGC. Another potential opportunity for investigators in the HLA diabetes community is participating in the MHC fine mapping initiative, which will use T1DGC samples to perform an extensive characterization of the 4 Mb spanning the HLA complex typing for 4500 SNPs, 63 msats, and HLA class I and class II genotyping. Both DNA samples and data can be obtained through the T1DGC Consortium Agreement (see policies at www.t1dgc.org). It was also deemed critical that future studies continue to rely on efficient core labs for msat/SNP typing and that strict control of DNA quality is enforced to ensure successful typing.

## Discussion

In this report, we detailed the major findings of the collaborative effort initiated under the auspices of the 13th IHW and reviewed the T1D-related workshop activities during the 14th IHIWS workshop held in Melbourne in 2005. This collaborative effort involved many laboratories worldwide and, based on the spirit of previous working groups and the available resources, focused on collecting samples from existing collections of patients, controls, and families. Confirmatory and new results were found showing heterogeneity of DR3 haplotypes, and further studies are likely to identify markers that help better quantify predisposition associated with DR3 haplotypes. Possible heterogeneity of the DRB1*1501 DQB1*0602 haplotype

was indicated for the first time, with significant reduction in the diabetes-protective effect typically associated with this haplotype, and follow-up studies are in progress. Other results using the eight msat loci to detect disease-predisposing loci in the HLA region additional to DR-DQ were extremely heterogeneous and demonstrated weak effects, for all three analysis methods: HPTDT, matched case–controls, and the haplotype method. Similarly, evidence of age of onset effects had moderate uncorrected *P* values and will require further confirmation. Nonetheless, the heterogeneity of the results is in itself an important result. Further, we have learnt a great deal about design of studies, which are useful for studying both the HLA region and the rest of the genome.

Our study particularly highlights the importance of a complementary, multistrategy array of methods to uncover all the different facets of complex genetic diseases. Also, previous work has shown that methods combining linkage and association can increase our power to detect significant effects, e.g. marker association segregation chi-square (MASC) (5), and further application of such approaches is eagerly awaited. The methods we have used in the study of the 13th IHW and HBDI data to detect additional disease-predisposing genes in the HLA region are the HPTDT (2, 12), matched genotype cases (2, 10) and controls, and the haplotype method (8, 11). The homozygous parent linkage test (9) has not been used, nor the single parent TDT (39, 40). However, while the haplotype method optimizes use of much more of the data, one cannot combine data across populations or haplotypes. Further, haplotype estimation is extremely problematic for diseases that do not show a mode of inheritance close to recessive. In this case, genotype frequencies will not be in Hardy–Weinberg equilibrium (17, 41), an assumption used in the EM algorithm to estimate haplotype frequencies. Fortunately, in the case of the HLA DR-DQ component to T1D, the genotype frequencies in patients are much closer to recessive than to dominant inheritance, so this is less of an issue than with dominant diseases such as ankylosing spondylitis and narcolepsy.

While the magnitude of the involvement of the HLA DR-DQ genes in T1D, their effects can be easily detected association and linkage studies even with small sample sizes. This is not the case with other HLA region genes and non-HLA genes. Large sample sizes and cross-ethnic studies are required to detect all HLA and non-HLA effects, including the complex hierarchy of predisposing through protective effects of the HLA DR-DQ haplotypes and genotypes. Because common HLA alleles are directly involved in disease, study of population-level variation complements disease studies. Issues of sample size were dramatically illustrated in application of the HPTDT (2) to the 13th IHW data, in both initial (13) and later analysis. Of the 433 families with a homozygous DR-DQ parent, 179 and 83, respectively were homozygous for DRB1*0301-DQB1*0201 and DRB1*0401-DQB1*0302. These numbers are exceedingly impressive and highlight the importance of collaboration and sharing. Nevertheless, to apply the HPTDT, the msat marker must be heterozygous. Further, because there are multiple msat alleles at each locus, the actual numbers in any one category quickly become small, even with the impressive sample size of the 13th IHW. The robustness of the HPTDT approach, and also the matched genotype case–control analyses, is attractive but is countered by the fact that many potential data are lost, and also one must be cautious that population-specific effects can lower power.

The results of the analysis on the 13th IHW and HBDI data presented at the 14th IHIWS also highlighted the critical importance of controlling for LD of markers with genes that were previously identified as susceptibility loci in that region, i.e. HLA DR-DQ in the case of T1D, especially when trying to identify additional susceptibility loci near loci that provide major contributions to disease risk. Choice of msats and/or SNPs in studies on disease is also crucial, both the number to be studied and their LD patterns with each other and primary predisposing loci. In the latter case, a new measure 'haplotype-specific heterozygosity' (HSH) (42) is very useful. Markers with high HSH are informative for detecting additional disease effects in a region. Further, the LD patterns on specific haplotypes, e.g. DR3 haplotypes for T1D, are also informative in choice of markers (27). The use of standard computer packages for haplotype estimation of HLA data can be problematic: HLA is too polymorphic for many programs, so that usually no more than five loci can be considered at a time. In some programs, only the most likely haplotype is listed, leading to bias. These same issues can apply to other analyses also and other specific issues: different programs can give different results for the same data set, because of handling of special cases, and how a program deals with rare haplotypes, or second affected sibs must always be considered in interpretation of results. The program PyPop (14, 15) was specifically designed to handle HLA data (it is equally applicable to other data of course) and gives overall population-level haplotype estimates; however, for studies on disease, it is not applicable to family data. The development of a standardized package for basic analysis of disease data (family and case/control) is well overdue.

The T1D component of the 13th IHW was the first organized effort to study the contribution of the extended HLA complex to T1D susceptibility using msat markers. This approach required a shift in the logistical organization of the study compared with previous working groups, including establishing investigators and laboratory cores that would coordinate the organization and performance of this effort with participating investigators willing to share samples and data. While the core group of investigators maintained closed contacts with participating investigators through frequent e-mail updates and some meeting

opportunities, this approach resulted in less direct involvement of study participants compared with earlier workshops. Identifying T1D susceptibility genes certainly requires large collaborative networks and centralized core laboratories, but it is also important to allow for greater involvement of participating investigators and the opportunity for more direct contributions to the study. The recently launched T1DGC, which is a much larger and more comprehensive effort, is built on this model. The T1DGC has appropriately developed expanded forms of communications with participating investigators, including regular meetings, as well as greater opportunity for investigators to access data and, importantly, DNA samples from renewable sources, for conducting independent studies. It is important to note that the 13th IHW T1D component has maintained contacts with the T1DGC, including collaborating on studying families that are also being studied by the T1DGC. Key investigators from the T1DGC actively participated in the 14th IHIWS in Melbourne, together with other investigators who helped review our knowledge about HLA and other genetic factors in T1D susceptibility. Potential areas of synergy and collaboration were also discussed. These include the utilization of T1D dbMHC as a shared data repository, a web-based database that was developed by NCBI together with 13th IHW investigators; the possibility of collaborating with the T1DGC to renew existing genetic material studied by the 13th IHW through whole genome amplification; and the opportunity to participate in further fine mapping studies of the HLA complex being launched by the T1DGC, an extremely important goal given that the HLA complex contributes ~50%–60% of the overall genetic risk of developing T1D.

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## Conflict of Interest Statement

All authors have declared no conflicts of interests.

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