

Receiver Operating Characteristic Analysis of HLA, CTLA4, and Insulin Genotypes for Type 1 Diabetes

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OBJECTIVE—This study assessed the ability to distinguish between type 1 diabetes-affected individuals and their unaffected relatives using HLA and single nucleotide polymorphism (SNP) genotypes.

RESEARCH DESIGN AND METHODS—Eight models, ranging from only the high-risk DR3/DR4 genotype to all significantly associated HLA genotypes and two SNPs mapping to the cytotoxic T-cell-associated antigen-4 gene (CTLA4) and insulin (INS) genes, were fitted to high-resolution class I and class II HLA genotyping data for patients from the Type 1 Diabetes Genetics Consortium collection. Pairs of affected individuals and their unaffected siblings were divided into a “discovery” ($n = 1,015$ pairs) and a “validation” set ($n = 318$ pairs). The discriminating performance of various combinations of genetic information was estimated using receiver operating characteristic (ROC) curve analysis.

RESULTS—The use of only the presence or absence of the high-risk DR3/DR4 genotype achieved very modest discriminating ability, yielding an area under the curve (AUC) of 0.62 in the discovery set and 0.59 in the validation set. The full model—which included HLA information from the class II loci DPB1, DRB1, and DQB1; selected alleles from HLA class I loci A and B; and SNPs from the CTLA4 and INS genes—increased the AUC to 0.74 in the discovery set and to 0.71 in the validation set. A cost-effective alternative is proposed, using genotype information equivalent to typing four SNPs (DR3, DR4-DQB1*03:02, CTLA-4, and INS), which achieved an AUC of 0.72 in the discovery set and 0.69 in the validation set.

CONCLUSIONS—Genotyping data sufficient to tag DR3, DR4-DQB1*03:02, CTLA4, and INS were shown to distinguish between subjects with type 1 diabetes and their unaffected siblings adequately to achieve clinically utility to identify children in multiplex families to be considered for early intervention.

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Type 1 diabetes is an autoimmune disease that gradually destroys insulin-producing β -cells. Immune interventions for type 1 diabetes are currently being attempted in prevention trials (1,2). Prevention trials assess the risk of type 1 diabetes in relatives based on circulating autoantibodies and evaluation of β -cell function using various methods. Although of clinical value, such dynamic biomarkers are relatively expensive to assay and are

sample dependent, so increasing attention is focused on the use of genetic data to predict the risk of disease in asymptomatic individuals (3).

Type 1 diabetes is one of the most widely studied complex genetic disorders, with genes in the HLA region reported to account for ~40–50% of the familial aggregation (4). The major genetic contribution from within the HLA region is attributable to the class II haplotypes encoded by the

DRB1, DQA1, and DQB1 loci (5), with independent risk also demonstrated at HLA-DPB1 and HLA-A, -B, and -C (6,7).

If HLA genotyping were replaced by minimal tag-single nucleotide polymorphism (SNP) genotyping, such a test would represent a cost-effective predictive value that could be applied from birth. In this study, we assess for the first time the ability to distinguish type 1 diabetes status in siblings (sibs) from families with type 1 diabetes based only on genetic information, using full high-resolution HLA genotyping for all classical loci and two SNP markers for the CTLA4 and INS genes. If combined with biomarkers for β -cell function, this test is expected to have utility in the selection of appropriate subjects for prevention trials.

RESEARCH DESIGN AND METHODS

Study subjects

The Type 1 Diabetes Genetics Consortium (T1DGC) is a large, worldwide collaborative study aimed at collecting and genotyping families with type 1 diabetes in a highly standardized fashion from multiple populations to aid in the search for additional type 1 diabetes genes within and outside the HLA region (8). An individual was designated as affected if he or she had documented type 1 diabetes with onset at age ≤ 37 years, had used insulin within 6 months of diagnosis, and had no concomitant disease or disorder associated with diabetes. Most patients came from families where more than one child was affected, and genotyping and clinical data were also collected for parents and unaffected sibs. High-resolution HLA genotyping was performed at eight classical HLA loci by four genotyping centers using standardized typing protocols, reagents, and quality-control procedures (9). In addition, the T1DGC HLA genotyping protocol, which used sequence-specific oligonucleotide-based linear arrays, also included assays for two type 1 diabetes-associated SNPs: the cytotoxic T-cell-associated antigen-4 gene (CTLA-4) +49 (A/G) (rs3087243) and insulin (INS) -23HphI (A/T) (rs689) (10). These

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two SNPs were selected for the receiver operating characteristic (ROC) curve analyses because their concomitant genotyping with *DRB1* in the T1DGC genotyping protocol assured that genotypes were available for all samples.

We selected data from families in the T1DGC final dataset with the following inclusion criteria: European ancestry, at least two affected sibs, and at least one unaffected sib. One affected sib and one unaffected sib were selected from each family ($n = 1,015$) to create the discovery set of affected/unaffected pairs; therefore, only families with DNA for unaffected sibs were used. In addition, 318 of the 1,015 pedigrees contained at least one additional unaffected sib. For these families, the second unaffected sib was paired with the second affected sib (not used for the discovery set), allowing the generation of a second set of 318 pairs of affected and unaffected sibs. These nonoverlapping pairs were used for validation of the risk models. For families with two affected/unaffected pairs, the pairs were randomly assigned to the discovery or validation sets. The discovery set consisted of 50.9% females, the mean age at onset of the affected sibs was 9.9 years (SD 7.8), and the mean age at recruitment was 22.2 years (13.2) for the unaffected sib and 22.7 years (12.7) for the affected sib. The validation set included 50.5% females, the mean age of onset was 9.3 years (7.05), and the mean age at recruitment was 22.6 years (14.2) for unaffected sibs and 23.3 years (14.1) for affected sibs.

Statistical methods

Allele selection and genotype coding.

The HLA alleles and genotypes that were selected for encoding type 1 diabetes risk were selected based on the literature reports for consistent associations with type 1 diabetes. Individuals were classified as 0 or 1, depending on the carriage or not of a given genotype as follows:

- DR3/DR4 only: Each individual was coded for the presence or absence of DR3/4 where DR3 = DRB1*03:01-DQB1*02:01 and DR4 = DRB1*04:01/2/4/5/8/13-DQB1*03:02 or *03:04 or *02:01.
- DRB1-DQB1 risk genotypes: For each individual, an indicator variable (taking values 0 or 1) was coded for if they carried one of the following genotypes: DR3/DR4, DR3/DR3, DR4/DR4, DR4/DRx, or DR3/DRx where DR3 = DRB1*03:01-DQB1*02:01, DR4 = DRB1*04:01/2/

4/5/8/13-DQB1*03:02 or 03:04 or 02:01, and x is any other haplotype, including DRB1*04:03 or any DRB1*04-carrying haplotypes that include DQB1*03:01.

- DRB1-DQB1 protective genotypes: Individuals were coded by variables (0 or 1) depending on carriage of one or two copies of DRB1*15:01-DQB1*06:02 (DR2_DQ6), DRB1*14:01-DQB1*05:03 (DR14), and DRB1*07:01-DQB1*03:03 (DR7_DQ9)
- DPB1: Carriage of alleles DPB1*04:02 (DP_0402) (protective) and of DPB1*03:01 (DP3) (susceptible) was included.
- Class I HLA-A and HLA-B: Carriage of risk alleles B*39:06 and A*24:02 was included.
- Polymorphisms in *INS* (rs689) and *CTLA4* (rs3087243): These SNPs were encoded as 0, 1, or 2 copies of the minor allele.

ROC analysis. The discriminating power of a given diagnostic is usually summarized by a ROC curve. In this type of analysis, subjects are ranked in descending order of their predicted risk, and the cumulative proportion of subjects who develop disease (case subjects) is plotted against the corresponding cumulative proportion of the population; that is, the sensitivity (true-positive fraction) is plotted in the y-axis versus 1-specificity (the false-negative fraction) in the x-axis (11). A perfect diagnostic would be represented by a line that starts at the origin and travels up the y-axis to 1 and then across the origin to an x-axis value of 1, thus having a total

area under the curve (AUC) of 1. A test with AUC of 0.5, which would be represented by a diagonal line from the x, y origin to 1 on both axes, has zero diagnostic value (11). A risk score was calculated for each individual using the logit equation:

$$\text{Logit} = \ln(p/1 - p) = \alpha + \beta_1 X_1 + \dots + \beta_i X_i$$

where p is the probability of the outcome (type 1 diabetes), α is the constant, and β is the natural logarithm value of the odds ratio for a specific predictor X_i . In this case, X_i is given by the presence or absence of specific genotypes or by the copies of alleles.

The discrimination ability of the models was examined in a test ($n = 1,015$ affected/unaffected sib pairs) and validation set ($n = 318$ pairs) using the PredictABEL package for R software (<http://cran.r-project.org/web/packages/PredictABEL/index.html>). All eight models were applied to the “discovery” and “validation” sets.

RESULTS—A logistic regression on type 1 disease status was fitted in the discovery set of 1,015 pairs of affected/unaffected sibs. Details for the eight models obtained are included in the Supplementary Data.

The AUC and the corresponding 95% CIs for each of these models were obtained in the discovery and validation sets (Table 1). To put these results in context, AUC values from 0.5 to 0.7 for a diagnostic/prognostic test represent

Table 1—AUC estimate (95% CI) for the performance of genetic markers in predicting type 1 diabetes using data from affected/unaffected sib pairs from the T1DGC

Model	Discovery set $n = 2,030$	Validation set $n = 636$	<i>P</i> value*
1. DR3/4 only	0.622 (0.604–0.64)	0.594 (0.562–0.627)	0.140
2. DR3/DR4 + <i>CTLA4</i> + <i>Ins</i>	0.657 (0.634–0.68)	0.625 (0.582–0.667)	0.194
3. <i>DRB1-DQB1</i> risk genotypes	0.711 (0.689–0.733)	0.683 (0.642–0.723)	0.234
4. <i>DRB1-DQB1</i> risk and protection genotypes	0.725 (0.703–0.747)	0.695 (0.655–0.735)	0.198
5. <i>DRB1-DQB1</i> + class I	0.729 (0.707–0.75)	0.701 (0.661–0.741)	0.227
6. <i>DRB1-DQB1</i> + class I + <i>DPB1</i>	0.732 (0.711–0.754)	0.704 (0.664–0.745)	0.231
7. <i>DRB1-DQB1</i> + class I + <i>DPB1</i> + <i>CTLA4</i> + <i>Ins</i>	0.739 (0.718–0.76)	0.712 (0.672–0.752)	0.241
8. <i>DRB1-DQB1</i> risk genotypes + <i>CTLA4</i> + <i>Ins</i>	0.721 (0.699–0.743)	0.694 (0.654–0.735)	0.251

**P* values are for the discovery vs. validation sets and were derived from a Z test using the mean and SE of both AUC values being compared. The full list of *P* values for the comparison of the AUCs of all eight models within the discovery and validation sets is provided in Supplementary Table 1.

low accuracy, values from 0.7 to 0.9 represent tests that are useful for some purposes, and values >0.9 represent diagnostic/prognostic tests with high accuracy (12).

The use of only the presence/absence of the high-risk DR3/DR4 genotype (model 1) achieves very modest discriminating results, yielding an AUC of 0.62 (95% CI 0.60–0.64) in the discovery set and 0.59 (0.56–0.63) in the validation set. Increasing levels of HLA risk (models 3, 4, and 5) and susceptibility genotypes progressively add to the AUC obtained to reach an AUC of 0.73 (0.71–0.75) in the discovery set and 0.70 (0.66–0.74) in the validation set for DRB1-DQB1 risk and protection genotypes, HLA class I, and HLA*DPB1 (model 6; Table 1). Full HLA genotyping plus *CTLA-4* and *INS* provided the highest discriminating model; the AUC was 0.74 (0.72–0.76) in the discovery set and 0.71 (0.67–0.75) in the validation set (model 7).

Notably, no significant difference in the AUC values achieved is observed among any of the models 3 to 7 (see Supplementary Table 1 for a full list of *P* values), but the AUC achieved by models 1 and 2 is significantly lower than that achieved by models 3 to 7, which include full modeling of the DR-DQ T1D risk, and not just the DR3/DR4 genotype, in both the discovery and validation sets, reaching *P* values as low as $P < 2.2 \times 10^{-16}$ for the comparison of the full model (model 7) with the only DR3/DR4 model (model 1) in the discovery set (Supplementary Table 1)

We found that minimal genotyping of DR3 and DR4-DQB1*03:02 genotypes plus *CTLA-4* and *INS* provided discriminating values of AUC of 0.72 in the discovery set and 0.69 in the validation set (model 8). This model is only marginally less informative, but not significantly so, than the full model of DRB1-DQB1 risk and protection genotypes + HLA class I + *DPB1* + *CTLA-4* + *INS* (Table 1) but requires considerably less genotype data.

CONCLUSIONS—In this study, we used the T1DGC data to investigate the discriminating performance of high-resolution HLA susceptible and protective genotypes, in combination with two additional validated genetic variants, to distinguish between type 1 diabetes patients and their affected sibs. We find that the use of genotypic information summarizing *HLA-DRB1-DQB1*, *DPB1*, *HLA-A*, and *HLA-B* alleles achieves discriminating levels of classification between affected

individuals and their sibs that can be of clinical utility (AUC >0.70). Because full HLA genotyping is currently too costly for large prevention trials, we have investigated the ROCs of a model that uses only information to derive the presence of combinations of the high-risk DR3 and DR4-DQB1*03:02 haplotypes in combination with the type 1 diabetes-associated SNPs for *CTLA-4* and *INS* that were typed concurrently with the HLA. Simplified SNP-based genotyping represents a plausible, cost-effective alternative to full HLA genotyping for this model. For example, a rapid test to identify DR3/DR4 subjects analyzed two SNPs, specifically, rs2040410 and rs7454108, which are associated with DR3-DQB1*02:01 and DR4-DQB1*03:02. These SNPs were analyzed in samples from 143 HLA-typed children who participated in the Diabetes Autoimmunity Study of the Young (DAISY) and in 5,019 subjects from the T1DGC. In the T1DGC samples, the two SNPs had a sensitivity of 98.5% (1,173 of 1,191) and a specificity of 99.7% for DR3/4-DQB1*03:02. In the DAISY population, the test was 100% sensitive and 100% specific (1).

Model 8 represents a minimal genotyping model of the presence or absence of the DR3- and DR4-DQB1*03:02-carrying genotypes, with appropriate weights for each genotype, together with the *CTLA-4* and *INS* SNPs. Model 8 achieved a substantial level of prediction (AUC = 0.72 in the discovery set and 0.69 in the validation set), comparable to model 7, which included full HLA genotyping data for multiple loci. A test such as model 8 would require genotyping of only four to six SNPs and might be further improved by the addition of SNP genotypes from other non-HLA, type 1 diabetes-associated loci (e.g., *PTPN22*, *IL2RA*, or *IFIH1*) (13) to provide a simple diagnostic test from a single sample that may be collected at birth.

The diagnostic may then be further improved by the use of dynamic biomarkers, such as autoantibodies and evaluation of β -cell function, to achieve optimal prediction. Indeed, a recent independent study has shown that among children who carry a high-risk DR-DQ genotype, the greatest diabetes discrimination was obtained by the sum of risk alleles for eight genes (*IFIH1*, *CTLA4*, *PTPN22*, *IL18RAP*, *SH2B3*, *KIAA0350*, *COBL*, and *ERBB3*) for progression from islet autoimmunity to type 1 diabetes (14).

Strengths of the current study include the large sample size, the use of internal

validation, and the first-time use of high-resolution HLA genotyping. Another important strength is the use of sibs of affected individuals matched for environmental, social, and lifestyle factors that may influence type 1 diabetes risk. Moreover, sibs of patients with type 1 diabetes represent a high-risk population that is commonly targeted by prevention trials.

The study has some limitations. The data used are cross-sectional rather than longitudinal, so the current study does not assess a risk for onset of diabetes over a given period of time. These data apply only to populations of European origin, and the discriminating value of HLA genotypes may not necessarily apply to other ethnic groups. Importantly, the data are derived from multiplex families where the genetic contribution to type 1 diabetes is particularly high and which are, therefore, primary targets for enrollment for prevention trials.

With these caveats in mind, the current study represents the first effort to systematically quantify the discriminating value of full HLA genotyping. In combination with autoantibodies, glucose, and C-peptide, data generated by this method may provide a high-accuracy prognostic tool for risk of type 1 diabetes in a high-risk population.

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A.M.V. carried out statistical analyses, researched data, and wrote the manuscript. M.D.V. and J.A.N. researched data, contributed to discussion, and reviewed and edited the manuscript. H.A.E. contributed to discussion and reviewed and edited the manuscript. A.M.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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