Models for Predicting Type 1 Diabetes in Siblings of Affected Children

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OBJECTIVE — To generate predictive models for the assessment of risk of type 1 diabetes and age at diagnosis in siblings of children with newly diagnosed type 1 diabetes.

RESEARCH DESIGN AND METHODS — Cox regression analysis was used to assess the risk of progression to type 1 diabetes, and multiple regression analysis was used to estimate the age at disease presentation in 701 siblings of affected children. Sociodemographic, genetic, and immunological variables were included in the analyses. Subanalyses were performed in a group of 77 autoantibody-positive siblings with additional metabolic data.

RESULTS — A total of 47 siblings (6.7%) presented with type 1 diabetes during the 15-year observation period. Young age, an increasing number of detectable diabetes-associated autoantibodies at initial sampling and of affected first-degree relatives, and HLA DR–conferred disease susceptibility predicted progression to type 1 diabetes. In the subgroup of 77 autoantibody-positive siblings, young age, HLA DR–conferred susceptibility, an increasing number of autoantibodies, a reduced first-phase insulin response, and decreased insulin sensitivity in relation to first-phase insulin response were associated with increased risk of progression to type 1 diabetes. Age at diagnosis was predicted by age, insulinoma-associated protein 2 antibody levels, and number of autoantibodies at initial sampling (R² = 0.76; P < 0.001). In the smaller cohort of autoantibody-positive subjects, first-phase insulin response and HLA DR–conferred susceptibility were additional predictors of age at diagnosis.

CONCLUSIONS — Information on autoantibody status and levels, HLA-conferred disease susceptibility, and insulin secretion and sensitivity seems to be useful in addition to age and family history of type 1 diabetes when assessing risk of progression to type 1 diabetes and time to diagnosis in siblings of children with newly diagnosed type 1 diabetes.

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Since the 1970s, several studies have indicated that HLA-conferred disease susceptibility and autoantibodies are useful in the prediction of type 1 diabetes among first-degree relatives of affected patients (1,2). Our purpose was to design predictive models for type 1 diabetes, integrating sociodemographic, genetic, immunological, and metabolic markers, and test their utility in prediction of type 1 diabetes in siblings of diabetic children. This approach is unique in that most earlier surveys presenting predictive models were based on relatively selected populations (3–5). Accordingly, assessing predictive strategies in an unselected sibling population is important and clinically relevant.

The contribution of autoantibodies in assessment of type 1 diabetes risk development is well established at the group level. It is also well known that HLA-conferred genetic susceptibility and a decreased first-phase insulin response (FPIR) to intravenous glucose increase risk. Assessment of future risk of type 1 diabetes has two dimensions. First, there is a need to have an estimate of the overall risk for subsequent development of clinical disease. Second, the family would like to know how soon a high-risk sibling of the first affected child might progress to type 1 diabetes. We decided to establish a two-step predictive strategy to 1) identify those siblings at highest risk for clinical disease and 2) assess the time frame within which a high-risk sibling will likely present with overt type 1 diabetes. Our aim was to generate clinically applicable predictive models for risk assessment of clinical diabetes in unaffected siblings of newly diagnosed type 1 diabetic children.

RESEARCH DESIGN AND METHODS — The study population was derived from the nationwide Childhood Diabetes in Finland (DiMe) study (6). The observation of the siblings was initiated shortly after the proband was diagnosed with type 1 diabetes. Blood samples were taken at intervals of 3–6 months during the first 2 years and at 6- to 12-month intervals during the following 2 years. Autoantibody-positive siblings were invited for further testing at an interval of 6–12 months to the end of 2002, whereas the testing of autoantibody-negative siblings ended after follow-up for the first 4 years. Only autoantibody data from the initial sampling were taken into account here. All the siblings were observed for progression to type 1 diabetes up to the end of year 2002, i.e., for an average period of 15.0 years (range 13.7–16.3). Observation of the siblings who progressed to clinical disease ended at diagnosis, which

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was based on clinical symptoms and an increased random blood glucose concentration (>10 mmol/l) or elevated fasting (>6.7 mmol/l) or random blood glucose on two occasions in the absence of symptoms (7).

Altogether, at least one blood sample was available from 758 siblings at the time of diagnosis in the index case. The present study cohort included all siblings with at least one serum sample for autoantibody analyses and data on HLA class II typing available. This resulted in a total series of 701 siblings with a mean age of 9.9 years (range 0.8–19.7). A total of 217 siblings were HLA DR3/DR4 heterozygous, 334 carried the DR4/non-DR combination, 97 carried the DR3/non-DR4 combination, and 53 had neither DR3 nor DR4. A total of 93 siblings tested positive for at least one diabetes-associated autoantibody, 49 being positive for a single autoantibody reactivity and 44 for multiple (two or more) antibodies. A total of 60 siblings tested positive for islet cell antibodies (ICAs), 20 for insulin autoantibodies (IAAs), 55 for GAD antibodies (GADAs), and 36 for insulinoma-associated protein 2 (IA-2) antibodies (IA-2As) at initial sampling. An intravenous glucose tolerance test (IVGTT) was performed in 77 of the 93 antibody-positive children.

**Disease-associated autoantibodies**

ICAs were determined with conventional immunofluorescence (8). The sensitivity of the ICA assay was 100% and the specificity 98% (9). IAAs, GADAs, and IA-2As were analyzed with specific radiobinding assays as described (10). The sensitivity of the IAA assay was 78% and the specificity 100% in the proficiency-testing program. The disease sensitivity of the GADA assay was 79% and the specificity 97% based on the 1995 Multiple Autoantibody Workshop (11). The corresponding characteristics of the IA-2A assay were 62 and 97%, respectively.

**IVGTT and the homeostasis model assessment of insulin resistance**

The IVGTTs were performed as described (12). Blood samples were taken before the glucose infusion and at 1, 3, 6, 10, 20, 30, 40, 50, and 60 min thereafter. Serum concentrations were measured radioimmunologically (13), and blood glucose levels were determined with the glucose oxidase method (14). The sum of the insulin concentrations at 1 and 3 min was defined as the FPIR to glucose. The glucose disappearance rate ($K_g$) was expressed as the percentage decrease in blood glucose per minute (%/min). FPIR levels $<45$ mU/l, a level that represents the third percentile of FPIR values in healthy control subjects (12), and $K_g$ values $<1.30$/min were considered abnormal. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the conventional formula: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5, as described previously (15,16). The conventional index correlated strongly ($r = 0.99$) with the newer HOMA computer model (17). Insulin resistance was related to insulin secretion by calculating the HOMA-IR/FPIR ratio.

**Genetics**

HLA DR typing was performed by conventional HLA serology as described (18). The HLA-conferred susceptibility was graded into four categories: no risk, HLA non-DR3/non-DR4; low risk, HLA DR 3/non-DR4; moderate risk, HLA DR4/non-DR3; and high risk, HLA DR3/DR4.

**Data handling and statistical analyses**

The data were evaluated statistically using cross-tabulation and χ² statistics for frequencies. Variables with a normal distribution were compared with the t test. The Mann-Whitney U test and nonparametric correlation analysis were applied when analyzing variables with a skewed distribution. The Cox regression analysis was used to assess factors associated with the risk of progression to type 1 diabetes, whereas multiple linear regression analysis was applied for the estimation of variables related to the age at diagnosis. The data initially included in the analysis of the total series of 701 siblings comprised the following potential predictors: age at first sampling, sex, HLA-conferred disease susceptibility (two or four categories), degree of HLA identity with the index case, initial autoantibody positivity and levels (ICAs, IAAs, GADAs, and IA-2As), age at diagnosis and sex of the index case, the number of children in the family, and the number of first-degree relatives affected by type 1 diabetes. In the smaller series comprising 77 autoantibody-positive siblings who had undergone an IVGTT, FPIR, Kg, HOMA-IR, and the HOMA-IR/FPIR ratio (natural logarithm transformed due to skewed distribution), were also included in the analyses. Cox regression analyses were performed with the Stata statistical software package version 8.0 (Stata, College Station, TX) and other statistical tests with the SPSS 11 software (SPSS, Chicago, IL). The proportionality of the hazards was checked by using log-cumulative hazard plots (Stata).

**RESULTS**

**Progression to clinical diabetes**

A total of 47 siblings (6.7%, 95% CI 5.0–8.8%) presented with clinical type 1 diabetes during the 15-year observation period. The mean age at the time of diagnosis was 13.9 years (range 1.4–28.4). Of the 47 progressors, 38 tested initially positive for at least one diabetes-associated autoantibody. Seven initially autoantibody-negative siblings seroconverted to antibody positivity before diagnosis. The risk of developing type 1 diabetes in the total series was associated with the age at first sampling, HLA DR–conferred disease susceptibility, the number of initially detectable diabetes-associated autoantibodies, and the number of affected family members (Table 1). Among the 77 autoantibody-positive siblings with metabolic data available, the age of the sibling, HLA DR–conferred susceptibility, the number of disease-associated autoantibodies, the FPIR, and the HOMA-IR/FPIR ratio turned out to be significant predictors of progression to type 1 diabetes (Table 1).

**The individual prognostic risk index**

Based on the Cox regression model, we calculated an individual prognostic risk index for each subject. We then performed a receiver operating characteristic (ROC) analysis to define a cutoff index leading to the best separation between progressors and nonprogressors. The cutoff index based on the total series was judged to be 0.25, resulting in a sensitivity of 78.7%, a specificity of 95.7%, and a positive predictive value of 56.9% for type 1 diabetes (Fig. 1). There were altogether 65 of 701 (9.3%) siblings with a prognostic index exceeding the cutoff value. Of these 65 siblings, 37 presented with clinical type 1 diabetes. The remaining 636 siblings (90.7%) had a prognostic risk index below the cutoff value of 0.25, and only 10 of them (1.6%) developed clinical type 1 diabetes. We compared the siblings below the cutoff value presenting with type 1 diabetes with those siblings who remained unaffected to assess factors predisposing to overt type 1 diabetes among these “protected” children. The progressors had higher GADA and IA-2A...
levels than the siblings who remained nondiabetic. In addition, they had initially more autoantibodies detectable and tended to be DR3/DR4 heterozygous more frequently than the unaffected siblings (data not shown). Among those who presented with type 1 diabetes, the siblings with an index in excess of 0.25 had a shorter duration of the preclinical period than those with a lower index (mean 4.9 ± 4.0 vs. 8.8 ± 3.3 years; P = 0.007). The prognostic index was inversely related to the duration of the preclinical period (rs = −0.40; P = 0.006). The predictive characteristics of a prognostic index >0.25 are compared with those of positivity for multiple (two or more) autoantibodies in Table 2.

**Prediction of age at diagnosis of type 1 diabetes**

The age at disease presentation was most effectively predicted with a linear regression model including age, IA-2A levels, and the number of initially detectable autoantibodies. This model explained ~76% of the variation in age at diagnosis (Table 3). When we applied this model on the 65 siblings with a prognostic index of >0.25, the observed age at clinical presentation was within the CI in 25 of the 37 progressors (68%), whereas all 28 non-progressors were predicted to present with diabetes before the end of the observation period.

The second model for the estimation of age at diagnosis including 77 siblings with metabolic data were based on the age of the sibling, the initial IA-2A level, HLA DR–conferred risk, and the initial FPIR value. This model explained 83% of the variation in age at diagnosis (Table 3). The application of this model on the 33 siblings with a prognostic risk index exceeding the cutoff value showed that the observed age at diagnosis was within the CI in all but 1 of the 25 progressors, but again all non-progressors were predicted to present with diabetes before the end of the observation period.

**CONCLUSIONS**

Although no effective modality for preventing or delaying progression to clinical type 1 diabetes has been recognized so far for clinical use in subjects at increased disease risk, there is still a rationale for establishing predictive models capable of identifying those individuals who are at the highest risk for developing type 1 diabetes and for estimating disease risk on an individual basis. Such a model will inevitably be needed as soon as the first treatment option modulating the pre-diabetic disease process has evolved. From a family point of view, the most urgent need is a reliable assessment of diabetes risk in unaffected siblings of children with newly diagnosed type 1 diabetes. Accordingly, the predictive model has to be based on information available or possible to generate within a limited time period close to the time of diagnosis in the index case. We have suggested that positivity for two or more autoantibodies seems to reflect a progressive irreversible

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**Table 1—Cox regression analysis for the estimation of risk for progression to clinical disease among 701 siblings of children with recently diagnosed type 1 diabetes and in a subgroup of 77 siblings with metabolic data available**

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>All siblings (n = 701)</th>
<th>Siblings positive for autoantibodies at baseline (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Age at first sampling (years)</td>
<td>0.83 (0.77–0.91)</td>
<td>0.76 (0.68–0.84)</td>
</tr>
<tr>
<td>Sex (boys vs. girls)</td>
<td>0.88 (0.49–1.55)</td>
<td></td>
</tr>
<tr>
<td>HLA-DR allele (high and moderate vs. low and decreased risk)</td>
<td>7.2 (2.8–18.2)</td>
<td>2.9 (1.1–7.6)</td>
</tr>
<tr>
<td>Antibody positivity (for diabetes-related autoantibodies ≥2 vs. 0–1)</td>
<td>55.9 (30.0–104.1)</td>
<td>54.1 (27.8–105.0)</td>
</tr>
<tr>
<td>Number of affected first-degree relatives (at the time of diagnosis in the index case ≥1 vs. 0)</td>
<td>3.3 (1.6–6.6)</td>
<td>3.2 (1.6–6.6)</td>
</tr>
<tr>
<td>FPIR (decreased vs. normal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (decreased vs. normal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR/FPIR (natural logarithm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are hazard ratios (95% CI). *Adjusted for all the other variables in column 2. †Adjusted for all the other variables in column 4.
autoimmune process, whereas positivity for only one type 1 diabetes–associated autoantibody appears to reflect harmless and even reversible β-cell autoimmunity (12,19,20). Now we have attempted to further refine the predictive model by integrating all data available on siblings of affected children close to the time of disease presentation in the index case. Based on our previous experience, we decided to aim at a two-stage model. The purpose of the first step was to assess overall risk for progression to clinical disease; the second step was to estimate the likely age at diagnosis of diabetes. We performed the risk assessment both in the total cohort including all available siblings and in a smaller series including those siblings who had additional metabolic markers available.

The strongest predictive model for progression to clinical disease in the total series included the age of the sibling at first sampling, HLA DR–conferred susceptibility, number of initially detectable autoantibodies, and the number of first-degree relatives with type 1 diabetes. We used the multivariate model to estimate the individual risk of a sibling for progression to overt type 1 diabetes by calculating an individual prognostic risk index in each sibling based on the Cox regression model. The optimal cutoff point was considered to be 0.25 based on a ROC analysis. Because the sensitivity was 79%, and the specificity of the model was as high as 96%, the prognostic risk index may provide a means for estimating individual risk. Of the 65 siblings with a risk index exceeding the cutoff value of 0.25, 37 (56.9%) developed type 1 diabetes. A total of 44 siblings tested initially positive for multiple (two or more) diabetes-associated autoantibodies in the total series, and 32 of these progressed to type 1 diabetes. Accordingly, the sensitivity of this risk marker was 68%, the specificity 98%, and the positive predictive value 73%. Although the prognostic index tended to be more sensitive than multiple autoantibody positivity, this difference remained nonsignificant, whereas the latter marker had significantly higher specificity (Table 2). Only when analyzing individuals who progressed to type 1 diabetes before the age of 16 years, the sensitivity of the prognostic index (93%) was higher than that of multiple autoantibody positivity (73%, difference 20%, 95% CI 2–38%). The observed predictive characteristics of the ROC cutoff value might, however, be too optimistic, since they are calculated based on the data on which the model was built. The inverse correlation between the prognostic index and the duration of the preclinical period indicates that a high index is a marker of a particularly aggressive disease process.

In the smaller series with metabolic data available, we found that both a reduced FPIR and an increased HOMA-IR/FPIR ratio reflecting a reduced insulin sensitivity relative to insulin secretion was associated with an enhanced disease risk. It is well established that a reduced early insulin response is associated with a high risk for progression to type 1 diabetes (3,4,12,21), whereas the observation that an increased HOMA-IR/FPIR ratio confers increased risk has been implicated by only one recent study (22). Fourlanos et al. (22) reported that autoantibody-positive first-degree relatives, who progressed rapidly to type 1 diabetes, were characterized by enhanced insulin resistance for their level of insulin secretion. Taken together, these observations suggest that the manifestation of clinical disease is affected by the balance between the insulin secretory capacity and peripheral insulin sensitivity.

When estimating the likely age at diagnosis in the total study cohort, we observed that age at initial sampling and the number of autoantibodies initially detectable were variables in common with the model predicting risk of progression to type 1 diabetes. The initial IA-2A level was the third parameter included in the model predicting age at disease presentation. IA-2As have been reported to appear in most cases as the last autoantibody during the pre-diabetic disease process (23,24), and they have also been observed to be the most predictive autoantibodies among first-degree relatives (10,25). The present observation stresses the role of IA-2As as predictive markers. The model was able to explain close to 80% of the variation in the age at diagnosis. The lack of HLA-conferred disease susceptibility from the model indicates that the pace of the pre-diabetic disease process is mainly regulated by factors other than the HLA class II genes (20). Approximately half of the observed ages at diagnosis in the 37 progressors were within the range of the CI of the estimations by this model among the 65 siblings with a prognostic index exceeding the cutoff value of 0.25, whereas all 28 nonprogressors were predicted to present with type 1 diabetes before the end of the observation period. Accordingly, this model for the prediction of age at diagnosis did not work precisely among the high-risk siblings.

The analysis of the series comprising 77 siblings with metabolic data available resulted in a model by which it was possible to explain ~83% of the variation in the age at diagnosis. This model included, in addition to age and IA-2A level at initial sampling, HLA DR–conferred disease susceptibility and early insulin response to intravenous glucose. It is intriguing that the genetic predisposition defined by HLA genes is included in this model but not in the model based on the total study cohort. One explanation could be that there is a strong correlation between the number of autoantibodies present and HLA-defined disease risk and that the inclusion of the former in the first model resulted in the exclusion of the latter from that model. The observation that there is an association between initial insulin secretory capacity assessed in terms of FPIR and age at diagnosis is consistent with our previous finding of a relation between the initial early insulin response and the time lag from first testing to type 1 diabetes presentation (12). Neither the fasting HOMA-IR index nor the HOMA-IR/FPIR ratio had any significant impact on age at diagnosis, suggesting that a reduced insulin sensitivity is a stronger determinant of disease risk than of progression rate to clinical diabetes. All but one of the observed ages at diagnosis were within the CIs of the estimated ages at disease presentation in the smaller series. This was at least partly a consequence of a substan-

Table 2—Predictive characteristics of a prognostic index >0.25 and positivity for multiple (two or more) autoantibodies

<table>
<thead>
<tr>
<th>Prognostic index &gt;0.25</th>
<th>Two or more Autoantibodies</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>78.7</td>
<td>68.1</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>95.7</td>
<td>98.2</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>56.9</td>
<td>72.7</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>98.4</td>
<td>97.7</td>
</tr>
</tbody>
</table>

Mrena and Associates
Prediction of type 1 diabetes in siblings

Table 3—Multiple regression analysis for the estimation of age at diagnosis in 47 siblings of affected children who contracted type 1 diabetes and in the 31 siblings with metabolic data available

<table>
<thead>
<tr>
<th>Siblings of affected children with type 1 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first sampling: 1.379; SE: 0.123; p &lt; 0.001</td>
</tr>
<tr>
<td>IA-2A level at first sampling: -0.039; SE: 0.012; p = 0.003</td>
</tr>
<tr>
<td>Initial number of autoantibodies: -0.649; SE: 0.415; p = 0.125</td>
</tr>
<tr>
<td>Intercept: 5.237; SE: 1.349</td>
</tr>
<tr>
<td>Fit of the model: R² = 0.76; F = 44.7; p &lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Siblings with metabolic data available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first sampling: 1.118; SE: 0.131; p &lt; 0.001</td>
</tr>
<tr>
<td>IA-2A level at first sampling: -0.021; SE: 0.011; p = 0.07</td>
</tr>
<tr>
<td>HLA DR-conferred risk*: 1.972; SE: 0.721; p = 0.011</td>
</tr>
<tr>
<td>FPIR: 0.0658; SE: 0.015; p &lt; 0.001</td>
</tr>
<tr>
<td>Intercept: -2.816; SE: 2.260</td>
</tr>
<tr>
<td>Fit of the model: R² = 0.83; F = 32.1; p &lt; 0.001</td>
</tr>
</tbody>
</table>

*Grading 0 (low risk) to 3 (high risk).

Initially greater SD in this model than that in the model based on the total study cohort. Again, the eight nonprogressors were predicted to develop type 1 diabetes before the end of the observation period, questioning the utility of this model for predicting age at diagnosis even when metabolic data are available.

Our work generated a novel approach for predicting type 1 diabetes with a multivariate model including the HOMA-IR/FPIR ratio as a measure of relative insulin resistance. The Cox regression model devised seemed to offer a feasible strategy for the identification of those siblings of children with newly diagnosed type 1 diabetes who will most probably progress to clinical disease. We think that this kind of information may be useful when the parents of a child with recently diagnosed diabetes are informed about the risk of clinical disease in their other children. The model for predicting age at diagnosis appeared to work well or satisfactorily among the true progressors but poorly among those who did not present with clinical disease. We think that this kind of information may be useful when the parents of a child with recently diagnosed diabetes are informed about the risk of clinical disease in their other children. The model for predicting age at diagnosis appeared to work well or satisfactorily among the true progressors but poorly among those who did not present with clinical disease. We think that this kind of information may be useful when the parents of a child with recently diagnosed diabetes are informed about the risk of clinical disease in their other children.

References
15. Cutfield WS, Jeffertes CA, Jackson WE,


