IDDM1 and Multiple Family History of Type 1 Diabetes Combine to Identify Neonates at High Risk for Type 1 Diabetes

Ezio Bonifacio, phd[1,2]
Michael Hummel, md[2]
Markus Walter, md[2]
Sandra Schmid, phd[2]
Anette-G. Ziegler, md[2]

OBJECTIVE — Children of affected probands are at increased risk for type 1 diabetes. The objective of this study was to determine and stratify the risk for islet autoimmunity and childhood diabetes in newborn offspring of affected parents using family history and HLA genetic markers.

RESEARCH DESIGN AND METHODS — Antibodies to islet autoantigens were measured at ages 9 months, 2 years, 5 years, and 8 years in 1,610 offspring of parents with type 1 diabetes participating in the German BABYDIAB study. HLA DR and DQ genetic typing was performed. Family history of type 1 diabetes was obtained from questionnaires.

RESULTS — Extensive family history of type 1 diabetes and HLA DR/DQ genotyping were associated with islet autoantibody and diabetes risks. Significant contributions to the child's risk for developing islet autoantibodies and type 1 diabetes were conferred by a multiple first-degree family history of type 1 diabetes (two parents or one parent and a sibling; adjusted hazard [HR] ratio, 6.2 for multiple islet autoantibodies and 7.8 for type 1 diabetes), high-risk HLA genotypes (adjusted HR, 11 and 10.9), and moderate-risk HLA genotypes (adjusted HR, 6.3 and 4.3) in a multivariate analysis. Combining these factors stratified the risk for islet autoantibodies from 1 to 46% and for type 1 diabetes from 0 to 19.5% by 5 years of age.

CONCLUSIONS — Risk of childhood diabetes in affected families can be stratified using a combination of genetic and family history markers very early in life.

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Type 1 diabetes is preceded by autoimmunity against the insulin-producing islet β-cells (1,2). The development of islet autoantibodies and type 1 diabetes is influenced by both genetic and environmental factors, and the detection of islet autoantibodies in members of affected families identifies a minority of individuals who have a markedly elevated risk of type 1 diabetes (3). On this principle, two large-scale intervention trials to delay onset of type 1 diabetes in islet autoantibody-positive first-degree relatives of patients with type 1 diabetes have recently been completed in North America and Europe (4,5). Despite promising pilot studies, both trials reported no delay in onset of type 1 diabetes in the treatment group. Successful prevention may, therefore, require alternative therapies and/or strategies that treat diabetes very early in the autoimmune process or before its appearance.

Primary prevention requires an ability to identify children who will develop autoimmunity. The German BABYDIAB study prospectively followed islet autoantibody and diabetes development in newborn offspring of parents with type 1 diabetes with the prospect of designing early intervention trials (6). The risk of developing islet autoantibodies in BABYDIAB children is strongly linked to HLA genes (7). Nevertheless, several children developed multiple antibodies and diabetes in the absence of established type 1 diabetes genetic markers, implying the presence of other strong familial genetic or environmental risk factors. In this study, we asked whether the extent of the family history contributed to the risk of developing islet autoantibodies and type 1 diabetes beyond that conferred by the locus of type 1 diabetes (*IDDM1*). The findings indicate that combining family history risk with known type 1 diabetes risk genes significantly improves our ability to predict the development of diabetes-associated autoantibodies in the neonate and to implement primary prevention trials in first-degree relatives of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

— BABYDIAB prospectively follows offspring of mothers and/or fathers with type 1 diabetes from birth. Venous blood sampling and collection of questionnaire data were performed at birth (cord blood) as well as at ages 9 months, 2 years, 5 years, 8 years, and 11 years (6–8). Recruitment into the study began in 1989 and ended in 2000. Offspring fulfilled entry criteria if they were recruited at birth and participated in the 9-month follow-up. A total of 1,610 offspring fulfilled these criteria, including 1,002 newborns of mothers with type 1 diabetes, 580 newborns of fathers with

From the [1]Immunology of Diabetes Unit, Istituto Scientifico San Raffaele, Milan, Italy; and the [2]Diabetes Research Institute and Academic Hospital Schwabing, Munich, Germany

Address correspondence and reprint requests to Prof. Dr. Anette-G. Ziegler, Institut für Diabetesforschung, Kölner Platz 1, D-80804 München, Germany. E-mail: anziegler@lrz.uni-muenchen.de.

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Abbreviations: GADA, GAD antibody; IAA, insulin autoantibody; IA2A, IA2 antibody; OGTT, oral glucose tolerance test; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

type 1 diabetes, and 28 newborns of two parents with type 1 diabetes. Islet autoantibodies were measured in samples from all scheduled visits and yearly after developing islet autoantibodies. Children were prospectively monitored for the development of diabetes. Monitoring in autoantibody-positive children was performed by oral glucose tolerance test (OGTT) every 6–12 months and/or monthly random blood glucose values. HLA DR and DQ genotypes were determined in 1,398 offspring, and the remaining 212 children did not provide a suitable sample for HLA typing. The median follow-up time from birth to last sample was 6.5 years (range 0.7–12.3) and from birth to last contact was 6.8 years (1.3–13.3). Offspring were followed for a total of 9,480 subject-years for islet autoantibodies and for 12,190 subject-years for development of type 1 diabetes. All families gave written informed consent to participate in the BABYDIAB study. The study was approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer No. 95357).

Autoantibodies

Insulin autoantibody (IAA), GAD antibody (GADA), and tyrosine phosphatase IA2 antibody (IA2A) were determined by radiobinding assays as described previously (6,9). The upper limits of normal corresponded to the 99th percentile of the control subjects and were 8.5 local units/ml or 25 World Health Organization (WHO) units/ml for GADA, 2.5 local units/ml or 4 WHO units/ml for IA2A, and 1.5 local units/ml for IAA (6,8). Using these thresholds for positivity, the assays had sensitivities and specificities of 84 and 96% (GADA), 66 and 100% (IA2A), 64 and 99% (IAA), and 78 and 100%, respectively, for multiple islet autoantibodies in the Third Diabetes Autoantibodies Standardization Program Proficiency Workshop. The interassay coefficient of variation for samples with low autoantibody titer was 11% for IAA, 18% for GADA, and 16% for IA2A. All measurements were performed on coded samples that were operator blinded.

IDDM1/HLA

HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles were typed using PCR-amplified DNA and nonradioactive sequence-specific oligonucleotide probes as described previously (7,10).

Family history of type 1 diabetes

At birth and at each follow-up visit, parents recorded which members in the family had type 1 diabetes, which for this purpose was defined as onset before 35 years of age and requiring insulin therapy.

Outcome definition

Outcome markers used in the study were the appearance of islet autoantibodies and the development of diabetes. Offspring were considered islet autoantibody positive if at least two samples after birth were found to contain one or more islet autoantibodies (IAA, GADA, or IA2A). Offspring who were found to have autoantibodies in only one follow-up sample were classified as islet autoantibody negative. Offspring with confirmed positive islet autoantibodies were subsequently classified as either single (only one autoantibody) or multiple (at least two autoantibodies) antibody positive. Diabetes was diagnosed according to WHO criteria (11). Diabetes was diagnosed by OGTT monitoring as part of the study protocol in 5 children and by random blood glucose measurements performed by families or pediatricians followed by OGTT or fasting blood glucose measurements in 19 children.

Statistical analysis

The cumulative frequencies of islet autoantibodies and type 1 diabetes were determined using life table analysis with follow-up calculated from birth until the age of the first positive sample or last negative sample as described previously (6). Cox's proportional hazards model was used to determine how the development of diabetes-associated islet autoantibodies and diabetes may be predicted in the neonate. Family history variables examined by univariate analysis in the model were the number of the child's parents or siblings with type 1 diabetes and whether the child's parents had a first-degree relative (mother, father, sister, or brother) with type 1 diabetes. Follow-up was calculated from birth until the age of the first positive sample for islet autoantibodies and from birth to the age at onset of diabetes or last contact for diabetes. Variables that were significantly associated with islet autoantibody risk ($P < 0.05$) were included in a multivariate analysis by Cox's proportional hazards model with forward conditional stepwise regression. The variable HLA genotype was also included in the multivariate analysis. HLA genotype was categorized according to previously de-

Table 1—*Risk for islet autoantibodies and type 1 diabetes in offspring stratified by family history*

	Multiple autoantibodies				Type 1 diabetes			
Family history variable (*n*)	Cases	5-year risk	HR	*P*	Cases	5-year risk	HR	*P*
First-degree type 1 diabetes family history of neonate								
Total	51				24			
One parent (1,565)	40	3.0 (2.1–3.9)	1*		17	0.8 (0.3–1.3)	1*	
Both parents, no sibling (28)	6	23.3 (6.4–40.2)	8.5 (3.6–20)	$<10^{-4}$	4	10.9 (0.1–22.8)	13.6 (4.6–40)	$<10^{-4}$
Parent and sibling (17)	5	30.4 (7.7–53.1)	13.5 (5.3–34)	$<10^{-4}$	3	11.8 (0.1–27.4)	19.2 (5.6–65)	$<10^{-4}$
Type 1 diabetes in first-degree relatives of child's parent†								
None (1,372)	39	3.3 (2.3–4.3)	1*		20	0.9 (0.4–1.4)	1*	
One or more (201)	10	5.6 (2.5–8.7)	1.8 (0.9–3.4)	0.08	4	1.8 (0.1–3.6)	1.4 (0.5–3.8)	0.5

Data are % (95% CI). *Reference cell used in Cox analysis for calculation of HR; †parents and siblings of child's parent (37 children were excluded because diabetes in the first-degree relatives of the parent was not defined as type 1 or type 2 diabetes).

Table 2—*Multivariate analysis of risk factors for multiple islet autoantibodies and type 1 diabetes*

Risk factor	Risk for multiple antibodies	*P*	Risk for type 1 diabetes	*P*
IDDM1				
High risk	11.0 (5.8–20.9)	<0.0001	10.9 (4.3–27.7)	<0.0001
Moderate risk	6.3 (2.8–14.4)	<0.001	4.3 (1.1–16.7)	0.03
First-degree type 1 diabetes history of neonate				
Multiple*	6.2 (3.1–12.2)	<0.0001	7.8 (3.1–19.4)	<0.0001

Data are HR (95% CI). *Both parents with type 1 diabetes or one parent and a sibling with type 1 diabetes.

fined diabetes risk in this study cohort (7) as high-risk DR4 (HLA DRB1*03/DRB1*04-DQB1*0302 or DRB1*04-DQB1*0302/DRB1*04-DQB1*0302), moderate-risk DR4 (DR4-DQ8/DRB1*08-DQA1*0401-DQB1*0402, DR4-DQ8/DRB1*13-DQA1*0102-DQB1*0604, DR4-DQ8/DRB1*01-DQA1*01-DQB1*0501), or other genotypes (7). For all analyses, a two-tailed *P* value of 0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS version 11.0, Chicago, IL).

RESULTS

Islet autoantibodies in BABYDIAB offspring

A total of 85 offspring developed islet autoantibodies during follow-up, 51 of which developed multiple islet autoantibodies (median age at first antibody-positive sample, 1.8 years). The remaining 34 islet autoantibody-positive offspring developed single antibodies only (median age at first antibody-positive sample, 5.2 years). Type 1 diabetes developed in 24 offspring, all of whom had islet autoantibodies before diabetes onset, 23 of whom had multiple antibodies, and none of whom had transient islet autoantibodies.

Family history and islet autoantibody/diabetes risk in offspring—univariate analysis

Risk for multiple antibodies was strongly associated with a multiple type 1 diabetes family history (Table 1). The risk for multiple islet autoantibodies in children who had one parent and no sibling with type 1 diabetes was 3.0% (95% CI 2.1–3.9) by 5 years of age. In comparison, risk was significantly increased in offspring who had both parents with type 1 diabetes (23.3% [6.4–40.2]; $P < 0.0001$) and in children with a parent and a sibling with type 1 diabetes (30.4% [7.7–53.1]; $P < 0.0001$). Among offspring who had only one parent with type 1 diabetes, the risk for multiple islet autoantibodies tended to be higher in offspring of fathers with type 1 diabetes (4.2% by 5 years of age [2.2–5.8]) than in offspring of mothers with type 1 diabetes (2.4% by 5 years of age [1.4–3.4]; $P = 0.06$; data not shown). Risk of developing multiple islet autoantibodies tended to be higher in children who had a parent with a first-degree family history of type 1 diabetes compared with those whose parents did not have a first-degree family history of type 1 diabetes (Table 1).

The risk of type 1 diabetes was significantly increased in offspring who had both parents with type 1 diabetes (10.9% by 5 years of age) and in offspring who had a parent and sibling with type 1 diabetes (11.8% by 5 years of age) compared with offspring with just one parent with type 1 diabetes (0.8% by 5 years of age; both $P < 0.0001$). The risk of developing single islet autoantibodies was not associated with multiple family history of type 1 diabetes and did not differ between offspring of mothers with type 1 diabetes and offspring of fathers with type 1 diabetes (data not shown).

Family history and genetic determinants of islet autoantibody and diabetes risk in offspring—multivariate analysis

Multiple family history was included in a multivariate analysis using Cox's proportional hazard model together with *IDDM1* (HLA) genotype (Table 2). Sequential addition of the variables using conditional stepwise regression showed that both HLA genotypes and a multiple family history of type 1 diabetes significantly contributed to the risk for multiple islet

Table 3—*Risk for islet autoantibodies and diabetes in offspring categorized by combinations of familial and IDDM1 risk factors*

Risk factor(s)	Single islet autoantibodies*	Multiple islet autoantibodies		Type 1 diabetes	
		Subjects	5-year risk	Subjects	5-year risk
None ($n = 1,095$)	24	11	1.0 (0.4–1.6)	4	0.3 (0.01–0.6)
One ($n = 287$)			9.1 (6.0–12.1)		3.3 (1.2–4.4)
Moderate-risk HLA ($n = 105$)	3	8	8.0 (2.7–13.3)	3	1.9 (0.1–4.5)
High-risk HLA ($n = 153$)	4	21	14.9 (8.9–20.9)	10	3.6 (0.6–6.6)
Multiple first-degree history (29)	1	4	15.3 (1.1–29.5)	3	6.9 (0.1–16.1)
Two ($n = 16$)			46.4 (22.4–68.8)		19.5 (0.1–39.5)
Moderate HLA, multiple FH ($n = 3$)	0	1	33.3 (0.1–87.7)	0	
High-risk HLA, multiple FH ($n = 13$)	0	6	48.7 (19.7–77.7)	4	23.4 (0.1–50)

Data are % (95% CI). *Two children with single islet autoantibodies are not included due to missing HLA genotype. FH, family history.

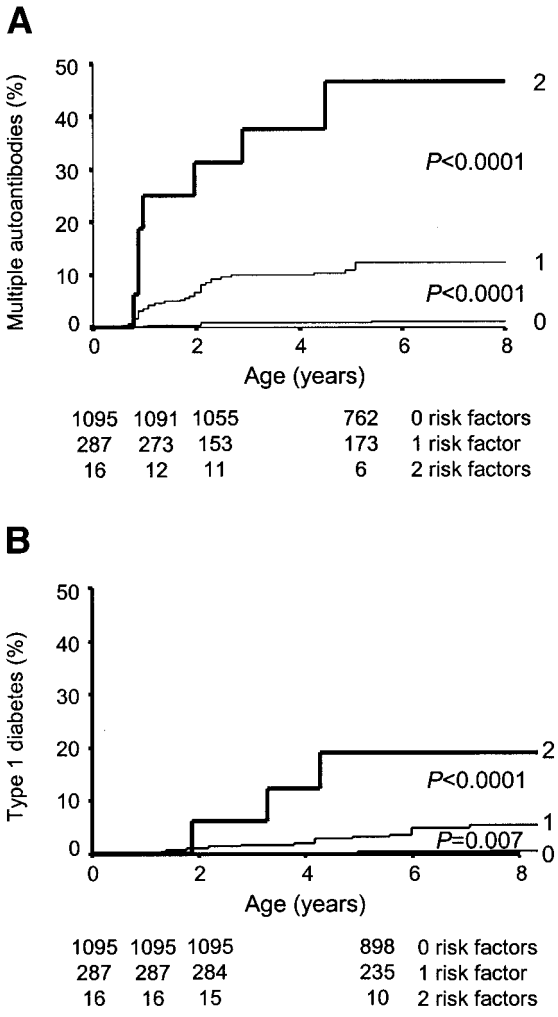

Figure 1—*Life table analysis of multiple islet autoantibody* (A) *and type 1 diabetes* (B) *development in BABYDIAB offspring according to* IDDM1 *and family history risk. The cumulative frequencies of multiple islet autoantibodies and type 1 diabetes are shown for the number of risk factors in the 1,398 children who had* IDDM1 *genotyping and family history data. Children are categorized as having no risk factors (thin line), one risk factor (thin line), or two risk factors (thick line) (high-risk or moderate-risk HLA genotypes, multiple first-degree family history of type 1 diabetes). Numbers under the abscissa indicate children remaining at follow-up in each category.*

autoantibodies. An increased risk for multiple islet autoantibodies was associated with high-risk HLA genotypes (adjusted hazard ratio [HR] 11; $P < 0.0001$), moderate-risk DR4 genotypes (6.3; $P < 0.001$), and a multiple first-degree family history of type 1 diabetes in the child (6.2; $P < 0.0001$). Although not significant in the univariate analysis, additional first-degree family history of type 1 diabetes in the child's parent conferred significant risk for multiple islet autoantibodies when added to this model ($P = 0.02$ for additional risk in model; adjusted HR 2.3 [1.2–4.5]). An increased risk of type 1 diabetes was associated with high-risk HLA genotypes (10.9; $P < 0.0001$), moderate-risk DR4 genotypes (4.3; $P = 0.03$), and a multiple first-degree family history of type 1 diabetes in the child (7.8; $P < 0.0001$).

Combining risk factors to identify neonates with high risk of type 1 diabetes

Multivariate analysis indicated that adding risk factors may help stratify risk. The ability to identify neonates who would develop islet autoantibodies or diabetes using both *IDDM1* (HLA) and a multiple first-degree family history in the child was therefore examined. After excluding 212 offspring because of a missing HLA genotype, at least one of these risk factors was identified in 303 of 1,398 children, including 40 of 51 children (78%) in whom multiple antibodies developed and 20 of 23 children (87%) in whom type 1 diabetes developed (Table 3). Four of the children who developed multiple antibodies and three of the children who developed diabetes had a multiple family history risk but no HLA risk genotype. The risk of developing multiple islet autoantibodies in children with one or more risk factor was 14.2% (10–18.4) by 5 years of age, compared with 1% (0.4–1.6) in children with no risk factors ($P < 0.0001$). The risk of developing multiple islet autoantibodies and type 1 diabetes was incremental with the number of risk factors present (Table 3 and Fig. 1). Children who had both an HLA risk genotype and a multiple family history of type 1 diabetes had risks of 46.4% (22.4–68.8) for multiple islet autoantibodies and 19.5% (0.1–39.5) for type 1 diabetes. This combination was present in 16 children, including 7 children (14%) in whom multiple antibodies developed and 4 children (17%) in whom diabetes developed later.

CONCLUSIONS — Young-onset type 1 diabetes is increasing in frequency in most western countries, particularly in children younger than 5 years (12–14). Children who have a first-degree relative with type 1 diabetes are at highest risk (2). Affected families, therefore, are targeted for counseling and for investigative studies to identify etiologic factors of type 1 diabetes and to test intervention therapies (4,5,8,15–22). The German BABYDIAB study was designed to determine and stratify the risk for islet autoimmunity and type 1 diabetes in offspring of parents with type 1 diabetes.

Having previously established that risk of diabetes was associated with the development of multiple islet autoantibodies at an early age (6), we asked whether development of multiple islet autoantibodies and diabetes could be predicted soon after birth. An ability to predict in which individuals multiple antibodies would develop could facilitate the design of targeted primary prevention such as the Trial to Reduce Type 1 Diabetes in the Genetically at Risk (23) or intensive follow-up studies such as The

Environmental Determinants of Diabetes in the Young Study, aimed at identifying etiologic factors in type 1 diabetes (24). We examined factors that could be determined soon after birth, including *IDDM1* (HLA) genotypes and the extent of the child's family history of type 1 diabetes. HLA loci provided much of the genetic susceptibility for developing multiple islet autoantibodies in the BABYDIAB cohort, but non-HLA factors were clearly visible. Markers of multiplex family history (additional sibling with type 1 diabetes, additional parent with type 1 diabetes) conferred risk on top of that conferred by *IDDM1* (HLA) in a multivariate model. The combination of these risk factors allowed identification of multiple antibody–positive children who did not have HLA risk genotypes, including two offspring with protective HLA genotypes who developed multiple autoantibodies and diabetes (data not shown). Several of the children who developed multiple islet autoantibodies also had an extended family history of type 1 diabetes in their parents. Offspring who remained single antibody positive had few of the genetic characteristics found in the multiple antibody offspring and in type 1 diabetes. Taken together, these findings are consistent with the hypothesis that familial factors other than HLA genes contribute to risk of type 1 diabetes.

The BABYDIAB study has shown that the likelihood of developing diabetes-relevant autoantibodies can be predicted using genetic markers in the neonate. A limitation of the study is that it only includes offspring of affected parents, and we cannot assume that the findings will be true in high-risk newborns of unaffected parents. In relatives, combining multiple type 1 diabetes family history and *IDDM1* (HLA) genotype could classify newborns into groups with multiple islet autoantibody risk ranging from 1 to almost 50% and with diabetes risk ranging from 0 to 20% by 5 years of age. Using this model, >75% of the children had a risk that was comparable to that of children in unaffected families and 1% of children had a risk approaching that in identical twins of type 1 diabetes patients. Neonates with the highest risk could be targeted for intensive prospective study to identify environment-gene interactions and could be candidates for prophylactic primary prevention therapy.

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