Islet-Specific Antibody Seroconversion in Patients With Long Duration of Permanent Neonatal Diabetes Caused by Mutations in the *KCNJ11* Gene

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Heterozygous activating mutations in the *KCNJ11* gene are a common cause of permanent neonatal diabetes (PNDM) (1,2). In contrast to the autoimmune type 1 diabetes, patients with *KCNJ11* mutations do not have serological markers of autoimmune β-cell destruction at disease onset (1,3–5). In such patients, hyperglycemia does not result from insulin-secreting cell destruction but rather from impaired insulin secretion. In addition, the majority of cases can be corrected with sulfonylurea therapy (2,6). Here, we report that carriers of the *KCNJ11* mutation that are immunonegative at onset may show presence of islet antibodies in the further course of the disease.

RESEARCH DESIGN AND METHODS

— We sought to evaluate the clinical and genetic characteristics of patients with neonatal diabetes in Poland, using the Nationwide Registry, which was established in 2005 (7,8). Automatic sequencing of the *KCNJ11* gene allowed identification of 15 patients with heterozygous mutations that cause neonatal diabetes. Sera from 11 carriers of the *KCNJ11* gene mutation were available for the present study. None of the patients had a family history of type 1 diabetes. Informed consent was obtained from all subjects or their parents. The study was conducted in accordance with the Declaration of Helsinki (revised in 2000) and accepted by the local ethics committee in Lodz, Poland.

β-Cell antibody analysis

At diabetes onset. Available data on islet antibody measurement at the onset of diabetes were limited. Five of our patients were diagnosed with diabetes before islet antibodies were implemented for clinical use. The rest of the obtained results come from several local laboratories. None of the tested patients had islet antibodies.

At study entry. Autoantibody analysis was performed in the Immunopathology Laboratory at the Department of Pediatrics, Medical University of Lodz, Poland, which is a reference laboratory for islet antibodies measurement and is a regular participant of international proficiency testing programs. The methods of antibody measurements were verified in the reference laboratory at the Barbara Davis Center for Childhood Diabetes in Denver, Colorado, with an inconsistency of 3% (courtesy of Prof. George Eisenbarth; see supplemental Figs. 1 and 2 for more information [available in an online appendix at http://dx.doi.org/10.2337/dc06-2440]).

Nonparametric statistics using the Mann-Whitney *U* test were applied to assess the differences between groups.

RESULTS

— Of 11 patients tested for the presence of islet antibodies, 5 were positive (Table 1). Among patients with >10 years' duration of neonatal diabetes, more than one-half showed the presence of at least one islet antibody. The relationship between disease duration and the occurrence of autoantibodies is shown in supplemental Fig. 2. Of five sera collected from the R201H mutation carriers, two (in subjects aged 13 and 50 years) were positive for islet antibodies. Moreover, two subjects with phenotypically more severe mutation, V59M, were negative for tested autoantibodies (Table 1). Thus, it is likely that the type of mutation is not related to the presence of autoantibodies. Despite detectable markers of islet-specific autoimmune process, all five patients responded well to sulfonylurea treatment, which indicates that a sufficient number of β-cells remained to maintain glucose homeostasis.

CONCLUSIONS

— Humoral markers of autoimmune type 1 diabetes are absent at onset of PNDM. This finding is consistent with all published studies on neonatal diabetes, due to mutation in the *KCNJ11* gene (1,3–5). In our patients' records, antibody measurement at disease onset were available for only five subjects, and, systematically, all patients were negative for β-cell–specific autoantibodies.

Our results demonstrate that immunonegative-at-onset carriers of the *KCNJ11* mutation may show seroconversion with long duration of the disease. It is known that apoptosis in β-cells can be elicited by various stimuli, including the perturbation of the metabolic and signal pathways (9–12).

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Received for publication 29 November 2006 and accepted in revised form 26 April 2007.

Published ahead of print at http://care.diabetesjournals.org on 2 May 2007. DOI: 10.2337/dc06-2440.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/dc06-2440.

Abbreviations: PNDM, permanent neonatal diabetes.

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

Table 1—*Detailed characteristics of the study group with autoantibody measurements*

	Diabetes duration (years)	Age at onset (months)	Mutation	Sex	Antibody at onset	Islet cell antibodies (N<5JDF)	GAD anitbody (N<9.1 IU/ml)	Insulin-associated protein 2 antibody (N<20 IU/ml)
Pol2	13	1	R201H	M	0	0	9.4	159.5
Pol9	20	3	V59M	F	NA	0	0.0	0.0
Pol11	22	2	K170N	F	NA	10	4.0	67.8
Pol13	35	2	R201H	F	NA	0	1.8	16.4
Pol14	21	3	R201H	F	NA	0	1.9	17.3
Pol16	5	1	V59M	M	0	0	0.0	0.0
Pol19	50	3	R201H	M	NA	40	1.1	20.8
Pol23	6	3	H46L	F	0	0	6.3	10.3
Pol31	11	2	E229K	M	0	10	3.6	7.6
Pol32	19	1	G53D	M	0	0	0.0	59.0
Pol33	11	2	R201H	F	0	0	0.2	0.0

F, female; M, male; N, normal value.

Gain-of-function mutations in Kir6.2 severely disturb metabolism of pancreatic β-cells, possibly promoting increased cell turnover. Recurrent exposure of tissue-specific antigens could lead to primary sensitization of immune cells. It appears that in this subgroup of patients, an autoimmune process may occur as a secondary effect to severe cell dysfunction, which results from *KCNJ*11 mutation.

All studies published to date that tested behavior of cells in the presence of mutated Kir6.2 focused on short-term effects. For instance, Lin et al. (13) did not observe a proapoptotic effect of mutated Kir6.2 in the insulin-secreting cell line INS-1 during their up to 1 week of experiments. Among patients with the *KCNJ*11 mutation, seroconversion occurs after at least a 10-year duration of PNDM. Therefore, it would be interesting to check the long-term effect of mutated Kir6.2 on the survival of insulin-secreting cells.

It has been reported that not all patients with PNDM caused by mutated Kir6.2 can transfer from insulin to sulfonylurea therapy (2). In some cases, it can be explained by the severity of mutation, as was demonstrated by in vitro studies (14). Interestingly, two diabetic mothers of two children also affected who responded well to sulfonylureas were resistant to this therapy. This observation shows that mutation type is not the only limiting factor in successful transfer to sulfonylureas and that diabetes duration may have an additional impact. Presence of islet antibodies proves that autoimmune response can be triggered in patients with mutated Kir6.2 protein. Ongoing processes may be responsible for the destruction of pancreatic islets, which in turn preclude treatment with sulfonylureas. Conversely, it is still speculative whether the autoimmune process could constitute an explanation for an observed lack of response to sulfonylurea therapy in some individuals with a long duration of PNDM.

Moreover, our finding is of clinical importance regarding type 1 diabetes diagnosis qualification versus type 2 or gestational diabetes. The presence of islet antibodies in patients with long duration of neonatal diabetes exemplifies that immune reaction against β-cells is not exclusively observed in type 1 diabetes. Therefore, diabetes diagnosis at age <6 months and lack of antibodies at the onset (but not in the later course of the disease) should constitute inclusion criteria for genetic evaluation of PNDM.

Acknowledgments— This study was supported by the Polish Ministry of Education and Science (Grants 2P0E13629 and 2P05E05129).

We thank Dr. Liping Yu from the Barbara Davis Center in Denver, Colorado, for islet antibodies evaluation and Prof. George Eisenbarth for critical discussion.

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