

Islet specific antibodies seroconversion in patients with long duration of permanent neonatal diabetes caused by mutations in the *KCNJ11* gene

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Running title: Anti-islet autoantibodies seroconversion in PNDM

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 beta cell destruction at the disease onset (1, 3-5). In those patients hyperglycemia does not result from insulin-secreting cells destruction, but from impaired insulin secretion, and in the majority of cases can be corrected by 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

Subjects

To evaluate clinical and genetic characteristics of patients with neonatal diabetes in Poland, the Nationwide Registry was established in 2005 (7, 8). Automatic sequencing of *KCNJ11* gene allowed identification of 15 patients with heterozygous mutations, which cause neonatal diabetes. Sera from 11 carriers of *KCNJ11* gene mutation were available for the present study. None of the patients has 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 as revised in 2000 and accepted by Local Ethical Committee in Lodz, Poland.

Beta cell antibodies(y) analysis

At the diabetes onset. Available data on islet antibodies measurement at the diabetes onset 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 tested patients had islet antibodies.

At entry of the present study. Auto-antibody analysis was performed in the

Immunopathology Laboratory at the Department of Pediatrics, Medical University of Lodz, which is a reference laboratory for islet antibodies measurement in Poland and is a regular participant of the international proficiency testing programs. The methods of antibodies measurements were verified in the reference laboratory at the Barbara Davis Center for Childhood Diabetes in Denver with 3% of inconsistency (by courtesy of Prof. George Eisenbarth, see appendix 1 and 2 for online-only publication).

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

Results

Out of 11 patients tested for the presence of islet antibodies 5 were positive (Table 1). Among patients with over 10-year duration of neonatal diabetes more than a half showed the presence of at least one islet antibody. Relationship between disease duration and the occurrence of autoantibodies is shown on Figure 2 (appendix 3 for online-only publication). Of 5 sera collected from the R201H mutation carriers two (age 13 and 50) were positive for islet antibodies. Moreover, two subjects with phenotypically more severe mutation, V59M, were negative for tested auto-antibodies (Table 1). Thus, it is likely that the type of mutation is not related to the presence of auto-antibodies. Despite detectable markers of islet-specific autoimmune process, all 5 patients responded well to sulfonylurea treatment, which indicates that sufficient number of beta 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 mellitus due to mutation in the *KCNJ11* gene (1, 3-5). In

our patients records, antibody measurement at onset were available for 5 subjects only and systematically all these patients were negative for beta 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). 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 seems that in this subgroup of patients autoimmune process may occur as a secondary effect to severe cell dysfunction resulting from *KCNJ11* mutation.

All studies published to date, which tested behavior of cells in the presence of mutated Kir6.2 focused on short term effect. For instance, Lin et al. did not observe proapoptotic effect of mutated Kir6.2 in the insulin-secreting cell line INS-1 during their up to one-week experiments (13). Among patients with the *KCNJ11* mutation, seroconversion occurs after at least 10-year duration of PNDM. It would therefore be interesting to check the long term effect of mutated Kir6.2 on survival of insulin-secreting cells.

It has been reported that not all patients with PNDM caused by mutated Kir6.2 could transfer from insulin to sulfonylurea therapy (2). In some cases it can be explained by the severity of mutation, as it was demonstrated

by *in vitro* studies (14). Interestingly, two diabetic mothers of two also affected children who responded well to sulfonylureas, were resistant to this therapy. This observation shows that a type of mutation is not the only limiting factor in successful transfer to sulfonylureas, and the 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 process may be responsible for destruction of pancreatic islets, which in turn precludes treatment with sulfonylureas. On the other hand, it is still speculative whether the autoimmune process could constitute an explanation for observed lack of response to sulfonylurea therapy in some individuals with long duration of PNDM.

Moreover, our finding is of clinical importance regarding to patient qualification as type 1 versus non-type 1 diabetic. Presence of islet antibodies in patients with long duration of neonatal diabetes proves that immune reaction against beta cells is not exclusively observed in type 1 diabetes. Therefore, diabetes diagnosis before 6 months of life and lack of antibodies at the onset but not in the later course of disease should constitute inclusion criteria for genetic evaluation of PNDM.

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Table 1. Detailed characteristics of the study group with auto-antibodies measurements

ID	Diabetes duration [years]	Age at onset [months]	Mutation	Sex	Ab at onset*	ICAs N<5JDF	GADA N<9.1 IU/ml	IA2-Ab 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
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
Pol17	22	2	K170N	F	NA	10	4.0	67.8
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

* NA – not applicable

Figure captions

Figure 1. (appendix 2 for online-only publication)

Immunofluorescence of the pancreatic islets of ICA positive individual with PNDM (Pol19). The endogenous insulin epitopes on the pancreatic sections were blocked with guinea pig polyclonal antibody against insulin before ICA visualization was performed.

Figure 2. (appendix 3 for online-only publication)

Duration of diabetes among anti-islet auto-antibodies positive (Ab+) and negative (Ab-) individuals with PNDM. Data presented as medians with interquartile range. All the differences were not statistically significant in nonparametric statistics.