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## Mutations in *IAPP* and *NEUROG3* genes are not a common cause of permanent neonatal/infancy/childhood-onset diabetes

Permanent/neonatal/infancy-onset diabetes is caused by defects in at least 10 different genes, including those encoding for insulin (*INS*) and the subunits of $K_{ATP}$ channel of the pancreatic B-cell (*KCNJ11, ABCC8*), which account for most of the cases diagnosed within 6 months of birth in international or national collections [1–3]. Interestingly, mutations in the *KCNJ11, ABCC8* and *INS* genes may be responsible for cases with diabetes onset in childhood and even adulthood [1,2,4–6]. In particular, we and other investigators have screened the *INS* gene in subjects who were diagnosed with diabetes during infancy/childhood, after selecting them for the absence of five [6] or two Type 1 diabetes autoantibodies [5]. These studies led to the identification of *INS* mutations in four subjects in whom diabetes was diagnosed between 3 and 13 years of age [5,6]. In addition, we found that *INS* gene mutations associated with infancy/childhood diabetes determine severe protein misfolding. As a result, mutant insulins are not secreted, causing sustained endoplasmic reticulum (ER)-stress, a process that eventually triggers apoptosis of pancreatic B-cells [2].

Human islet amyloid polypeptide (IAPP) is abundant in the pancreatic B-cell and is co-secreted with insulin [7]; therefore, we hypothesized that mutations in *IAPP* may cause diabetes in infancy or childhood with a mechanism similar to that described for insulin mutations. Transcription factors required for endocrine pancreas development and/or B-cell specification, such as neurogenin3, are also potential candidates for monogenic diabetes. Neurogenin3 (*NEUROG3*) gene expression co-localizes with insulin, glucagon and insulin promoter factor 1 (IPF1) in the early stages (8–12 weeks of fetal age) of the developing human endocrine pancreas [8] and *Ngn3* knock-out mice lack endocrine cells in the pancreas and die as a result of diabetes a few days after birth [9]. Of note, mutations of *NEUROG3* have been detected recently in three patients with a genetic form of secretory diarrhoea; interestingly, two of these subjects also developed non-autoimmune diabetes at the age of 8 years [10]. The aim of our work was to look for mutations in the *IAPP* and *NEUROG3* genes in patients with diabetes diagnosed in infancy/childhood who were negative for at least two Type 1 diabetes autoantibodies.

We studied four patients who were negative for five Type 1 diabetes autoantibodies [islet cell antibodies (ICA), glutamic acid decarboxylase antibodies (GADA), insulinoma-associated-2 autoantibodies (IA-2A), insulin autoantibodies (IAA), zinc transporter-8 antibodies (ZnT8A)] [6], two patients who were negative for four autoantibodies (ZnT8A not tested), two who were negative for three autoantibodies (ICA and ZnT8 not tested) and two who were negative for IA-2A and GADA. Sera from the last six patients were not available for assessment of the full panel of autoantibodies. All these probands were negative for *KCNJ11* and *INS* genes mutations. The age range of diabetes onset was 7 months to 10 years. In addition, we screened three *KCNJ11-INS-ABCC8*-negative patients with diabetes onset < 6 months after birth. Direct DNA sequencing of the three exons and intron–exon boundaries of the *IAPP* gene and of the coding exon 2 of the *NEUROG3* gene did not identify any pathogenic mutations. However, we did detect two described variants of *NEUROG3*: G167R (p.Gly167Arg c.499G > A) and F199S (p.Phe199Ser c.596T>C). Previously it has been shown that neither the G167R nor the F199S variants are associated with maturity-onset diabetes of the young (MODY) in Japanese [11] or Danish subjects [12]. In addition, no differences in allele frequencies between glucose-tolerant individuals and patients with Type 2 diabetes have been found [12]. We also examined the *NEUROG3* G167R and F199S variants in 155 normal Italian control subjects and detected no difference in minor allele frequencies from patients studied in this report (Table S1, available online).

We conclude that mutations of *hIAPP* and *NEUROG3* genes are not a common cause of neonatal/infancy/childhood-onset diabetes.

## Competing interests

Nothing to declare.

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## Supporting information

## Insulin resistance and endothelial dysfunction in type 2 diabetic patients with non-alcoholic steatohepatitis

Nonalcoholic steatohepatitis (NASH) is commonly associated with the clinical features of the metabolic syndrome such as obesity, type 2 diabetes mellitus and dyslipidaemia. Thus it may possibly be associated with the development of insulin resistance and atherosclerosis. Endothelial dysfunction is thought to be an important early feature in the development of atherosclerosis and insulin resistance. It is unknown, however, whether the presence of NASH facilitates the development of insulin resistance and endothelial dysfunction in type 2 diabetic subjects. In this study, we investigated the degree of endothelial dysfunction and insulin resistance in type 2 diabetic patients with or without NASH.

We recruited type 2 diabetic patients with and without NASH in the Department of Diabetes and Metabolic Diseases of the Osaka General Medical Center and Department of Diabetes and Atherosclerosis of the National Cardiovascular Center. In all subjects with NASH, levels of liver enzymes were chronically elevated, and hepatic steatosis was observed on ultrasonography. The diagnosis for NASH was based on liver biopsy and exclusion of other known aetiologic factors of chronic liver disease (alcohol abuse or intake more than 20 g/day, viral hepatitis, autoimmune hepatitis, and use of hepato-toxic drugs). The histological criteria of NASH and the score of necroinflammatory grade, fibrosis stage, and steatosis grade on liver biopsies were based on a previous report [1]. For ethical reasons, liver biopsy was performed only in patients with chronically elevated levels of liver enzymes and hepatic steatosis on ultrasonography. Based on the results of the liver biopsy, ten subjects were diagnosed as having NASH. Further analyses regarding the association between histological criteria such as necroinflamation grade, fibrosis stage, and steatosis grade were performed only for the patients with NASH.

To examine the relationship between NASH and insulin resistance, we estimated insulin resistance using the steady state plasma glucose (SSPG) method as reported previously [2]. To examine the relationship between NASH and endothelial cell dysfunction, we estimated endothelial function using the values of flow-mediated vasodilation (FMD) as described previously [2]. Liver biopsy was performed on the day of the admission, and on the next day, after overnight fasting, %FMD values and SSPG levels were estimated. In addition, to examine the relationship between inflammation grade/ fibrosis stage/ steatosis grade and insulin resistance/ endothelial dysfunction, we divided the subjects into two groups based on the inflammation grade, fibrosis stage, and steatosis grade determined by liver biopsy. Values are expressed as means ± SE. Statistical analyses were performed using the Student's $t$-test. A value of $P < 0.05$ was considered to be significant. The study protocol was approved by the ethics committee of the Osaka Prefectural General Hospital and National Cardiovascular Center.

Type 2 diabetic patients with NASH ($n = 10$) and without NASH ($n = 15$) of mean age $58.1 \pm 2.0$ and $58.7 \pm 2.1$ years, BMI $26.7 \pm 0.9$ and $27.0 \pm 0.5$ kg/m$^2$, AST $69 \pm 6$ and $18 \pm 2$ units/l ($P < 0.05$), ALT $102 \pm 11$ and $16 \pm 2$ units/l ($P < 0.01$), $\gamma$-glutamyl transferase $79 \pm 10$ and $39 \pm 9$ units/l ($P < 0.01$), HbA$_{1c}$ $7.1 \pm 0.4$ and $7.2 \pm 0.2\%$, total cholesterol $4.9 \pm 0.3$ and $4.9 \pm 0.2$ mmol/l, triglycerides $1.4 \pm 0.17$