Running Title: Monogenic mutations and type 1 diabetes

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Comprehensive screening for monogenic diabetes in 89 Japanese children with insulin-requiring antibody-negative type 1 diabetes

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Word count, 2,763; Tables 4; Figure 1

ABSTRACT

Background: Mutations in causative genes for neonatal diabetes or maturity-onset diabetes of the young have been identified in multiple patients with autoantibody-negative type 1 diabetes (T1D).

Objectives: We aimed to clarify the prevalence and phenotypic characteristics of monogenic abnormalities among 89 children with autoantibody-negative insulin-requiring T1D.

Methods: Mutations in 30 genes were screened using next-generation sequencing, and copy-number alterations of four major causative genes were examined using multiplex-ligation dependent probe amplification. We compared the clinical characteristics between mutation carriers and non-carriers.

Results: We identified 11 probable pathogenic substitutions (six in *INS*, two in *HNF1A*, two in *HNF4A*, and one in *HNF1B*) in 11 cases, but no copy-number abnormalities. Only two mutation carriers had affected parents. *De novo* occurrence was confirmed for three mutations. The non-carrier group, but not the carrier group, was enriched with susceptible HLA alleles. Mutation carriers exhibited comparable phenotypes to those of non-carriers, except for a relatively normal body mass index (BMI) at diagnosis.

Conclusions: This study demonstrated significant genetic overlap between autoantibody-negative T1D and monogenic diabetes. Mutations in *INS* and *HNF* genes, but not those in *GCK* and other monogenic diabetes genes, likely play critical roles in children with insulin-requiring T1D. This study also suggests the relatively high *de novo* rates of *INS* and *HNF* mutations, and the etiological link between autoimmune abnormalities and T1D in the non-carrier group. Carriers of monogenic mutations show nonspecific phenotypes among all T1D cases, although they are more likely to have a normal BMI at diagnosis than non-carriers.

KEY WORDS: *INS*, *HNF*, mutation, next-generation sequencing

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INTRODUCTION

Diabetes mellitus is classified into type 1 (T1D), type 2, other specific types, and gestational diabetes (1, 2). T1D is subdivided into type 1A associated with autoantibodies against glutamic acid decarboxylase 65 (GADA), tyrosine phosphatase-like insulinoma antigen 2 (IA2A), insulin (IAA), islet cells (ICA), or β-cell-specific zinc transporter 8 (ZnT8A), and type 1B that occurs independently of these autoantibodies (1, 2). The group of "other specific types of diabetes" includes neonatal diabetes and maturity-onset diabetes of the young (MODY), both of which arise from monogenic mutations. Previous studies have identified 24 genes, including *INS*, *KCNJ11*, and *ABCC8,* that jointly account for most cases of neonatal diabetes, and 13 genes, including *GCK*, *HNF1A*, and *HNF4A*, whose mutations and deletions are present in a substantial fraction of MODY cases (3, 4, 5, 6, 7). Seven genes are involved in both neonatal diabetes and MODY.

Patients with monogenic diabetes are usually distinguishable from those with other types of diabetes, because neonatal diabetes is characterized by an early disease onset within the first few months of life, and MODY represents autosomal dominant diabetes with partially preserved insulin secretion (1, 3). Nevertheless, mutations in the monogenic diabetes genes have been identified in multiple patients with childhood-onset autoantibody-negative T1D (8, 9, 10, 11, 12). We, the Japanese Study Group of Insulin Therapy for Childhood and Adolescent Diabetes (JSGIT), previously performed Sanger sequencing-based mutation analysis of *INS* and *KCNJ11* on 34 patients with early onset (≤ 5 years of age) autoantibody-negative diabetes, and identified *INS* and *KCNJ11* mutations in five cases and one case, respectively (13). Similarly, Sanger sequence analysis of *INS, KCNJ11, HNF1A*, and *HNF4A* on 32 Japanese patients detected *INS* and *HNF1A* mutations in three cases (14).

Most recently, Johansson et al. performed the first comprehensive mutation screening of MODY genes on a large cohort of children with autoantibody-negative

diabetes. The authors studied 469 affected children by next-generation sequencing (NGS) and identified probable damaging variants of *GCK*, *INS*, and *HNF* genes in 6.5% of the cases. These findings provided evidence for a significant association between T1D and monogenic mutations. However, because the subjects of Johansson et al. included patients of various clinical severities, the frequency of monogenic mutations in patients with insulin-requiring T1D remains to be determined. Furthermore, NGS may miss pathogenic copy-number variations (CNVs), although deletions involving *HNF1A*, *HNF1B*, and *GCK* have been identified in a few patients with MODY (15).

Here, we conducted a NGS-based mutation screening of 30 monogenic diabetes genes in 89 Japanese children with insulin-requiring autoantibody-negative T1D. We also analyzed CNVs involving *GCK, HNF1A, HNF4A,* and *HNF1B* using multiplex-ligation dependent probe amplification (MLPA). The clinical characteristics of mutation carriers were compared to those of non-carriers.

METHODS

Participants

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the participants or their parents. We enrolled 89 unrelated Japanese children clinically diagnosed with T1D (Tables 1, 2, and S1). All participants required persistent insulin therapy and satisfied the following criteria: (i) recruited by JSGIT between January 2008 and June 2013; (ii) diagnosed with T1D based on the criteria of the World Health Organization published in 1998 (16); (iii) diagnosed between the age of 0.5 and 16.0 years; (iv) had detailed medical records including data of height and weight at diagnosis; and (v) showed negative results for all diabetes-associated autoantibodies examined. In all cases, GADA had been tested at diagnosis, and other autoantibodies were also examined in several cases (Tables 2 and S1). The participants included 47 children who were previously subjected to Sanger sequencing-based mutation analysis (13, 14).

Molecular analyses

Genomic DNA was extracted from peripheral blood samples of the participants. To detect nucleotide substitutions, we designed an NGS panel (HaloplexHS; Agilent Technologies, Santa Clara, USA) targeting the coding- and non-coding exons and their flanking regions of 30 genes known to cause monogenic diabetes (*ABCC8, BLK, CDKN1C, CEL, EIF2AK3, FOXP3, GATA4, GATA6, GCK, GLIS3, HNF1A, HNF4A, HNF1B, IER3IP1, INS, KCNJ11, KLF11, MNX1, NEUROD1, NEUROG3, NKX2-2, PAX4, PDX1, PTF1A, RFX6, SIRT1, SLC2A2, SLC19A2, SLC29A3*, and *ZFP57*). The total amplicon number was 11,033 and the target size was 205.8 kb with a theoretical coverage of 98.5% for the targeted regions. According to the manufacture's protocol, individually indexed HaloplexHS libraries were prepared, and sequenced on a HiSeq

(Illumina, San Diego, USA). Base calling, read filtering, and demultiplexing were performed with the standard Illumina processing pipeline. We used BWA 0.7.5 to map reads against the human reference genome (build: hg19) with the default settings. Local realignment, quality score recalibration, and variant calling were performed with GATK3.6 using the default setting. We used ANNOVAR for annotation of the called variants.

All non-synonymous substitutions and nucleotide changes at a splice site were evaluated by database search [the Exome Aggregation Consortium Browser (ExAC, http://exac.broadinstitute.org); the 1000 Genomes Database (http://www.ncbi.nlm.nih.gov); the Human Genetic Variation Database (HGVD, http://www.hgvd.genome.med.kyoto-u.ac.jp) and the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk)]. The functional consequences of missense substitutions were predicted by Sorting Intolerant From Tolerant (SIFT, http://provean.jcvi.org/genome_submit_2.php); PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/); Mutation Taster (http://mutationtaster.org/); the Combined Annotation Dependent Depletion (CADD, http://cadd.gs.washington.edu); and the Mendelian Clinically Applicable Pathogenicity (M-CAP, http://bejerano.stanford.edu/mcap/index.html)]. Substitutions previously identified in patients with diabetes were classified as pathogenic. Nucleotide changes whose frequency in the general population was ≥ 0.001 and were predicted as benign by more than three of the five *in silico* analysis were excluded as probable benign variants. All variants of interest were confirmed by PCR-based Sanger sequencing. When possible, we analyzed parental DNA samples of mutation-positive participants.

To detect CNVs involving *GCK, HNF1A, HNF4A,* or *HNF1B*, we performed MLPA analyses using the SALSA MLPA MODY mix-1 probemix (catalog number, P241; MRC-Holland, Amsterdam, The Netherlands).

HLA alleles and clinical characteristics of mutation carriers

We genotyped HLA-DRB1 using the Luminex Multi-Analyte Profiling system with the WAKFlow HLAtyping Kit (Wakunaga, Hiroshima, Japan). We determined known diabetes susceptible alleles (*09:01, *04:05, *08:02) and protective alleles (*15:02, *15:01, *08:03, *04:06) in the Japanese population (17). The difference in the frequencies of susceptible and protective alleles among mutation carriers and non-carriers were analyzed. We also compared the frequencies of susceptible and protective alleles between mutation carriers and the Japanese general population [the Database of the HLA laboratory (http://hla.or.jp/)]. In this analysis, the brother of patient 5 who had diabetes and the same mutation as the proband was included in the group of mutation carriers.

In addition, we compared phenotypic characteristics between mutation carriers and non-carriers, and between *INS* mutation carriers and carriers of other mutations. The body mass index (BMI, weight / height^2) SD was calculated based on the data of the Japanese population (18). We examined fasting blood C-peptide values at diagnosis. Cases with C-peptide values less than 0.6 ng/mL were considered as having endogenous insulin deficiency (19). Diabetic ketoacidosis (DKA) was diagnosed according to the Clinical Practice Consensus Guidelines of the International Society for Pediatric and Adolescent Diabetes (ISPAD) (20). In addition, we examined whether mutation carriers had extra-pancreatic complications.

The statistical significance in the differences between two participant groups was analyzed using the Fisher's exact test for categorical variables, and the Mann–Whitney U test for continuous variables. A-two tailed *p*-value with an alpha level for significance was determined as ≤ 0.05. All statistical analyses were performed using the EZR system (version 1.32, http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedOSX.html), a graphical user interface for R (21).

RESULTS

Molecular analysis

Eleven probably pathogenic sequence variants were identified in 11 patients (patients 1–11; Tables 2 and S2 and Figure 1). All variants were present in heterozygous states. The variants consisted of six missense substitutions in *INS* (p.C31Y, p.V42A, p.G75C, p.R89C, p.C96F, and p.C96R), two in *HNF1A* (p.R131Q and p.R203S), two in *HNF4A* (p.Q142H and p.E256A), and one in *HNF1B* (p.L168P). Of these, p.G75C and p.C96F in *INS,* p.Q142H and p.E256A in *HNF4A*, and p.L168P in *HNF1B* were hitherto unreported, while the remaining six substitutions have previously been identified in patients with diabetes (13, 22, 23, 24, 25). Notably, mutation p.G75C is located in the C-peptide sequence. Nine of 11 variants were not found in the public databases, whereas p.R131Q in *HNF1A* and p.E256A in *HNF4A* have been submitted to the ExAC Browser as an extremely rare nucleotide change (allele frequency, 1/121,198 and 1/121,396). Three substitutions in *INS* (p.C31Y, p.C96F, and p.C96R) affect cysteine residues involved in disulfide bond formation, and p.V42A disrupts an amino acid next to the cysteine residue at the 41st codon (26). In addition, p.R89C is known to cause proinsulin misfolding (26), while the effect of p.G75C remains to be clarified. The variants in *HNF1A*, *HNF4A*, and *HNF1B* are invariably located within functionally important domains (27, 28, 29, 30) (Figure 1). Furthermore, p.Q142H in *HNF4A* affects the last nucleotide of exon 4. Parental analysis revealed that three *INS* mutations (p.V42A, p.G75C, and p.R89C) were *de novo*, while p.C31Y of patient 1 and p.C96F of patient 5 were inherited from their parent with diabetes. Parental samples of other patients were unavailable for genetic analysis.

MLPA detected no copy-number alterations of exons of *GCK, HNF1A, HNF4A,* or *HNF1B*.

HLA alleles and clinical characteristics of mutation carriers

We compared the HLA-DRB1 allele frequencies and clinical information between mutation carriers (n = 12) and non-carriers (n = 78) (Table 3). Susceptible HLA-DRB1 alleles were less frequent in the carrier group than the non-carrier group (4/24 vs. 96/156, p = 0.00004), while protective HLA-DRB1 alleles were more frequent in the carrier group (6/24 vs. 8/156, p = 0.004). The frequency of susceptible HLA-DRB1 alleles in the carrier group was slightly lower than that in the Japanese general population (10,235/31,973, p = 0.01), and the frequency of protective HLA-DRB1 alleles were comparable between these two groups (9585/31,973 in the general population, p = 0.43).

Clinical features were almost comparable between the two groups, except for the median BMI SD scores at diagnosis, which were normal in mutation carriers and slightly reduced in non-carriers (mean SDS, 0.0 vs. -0.8, p = 0.02). In particular, endogenous insulin deficiency indicated by low C-peptide levels were observed in both carriers and non-carriers (1/5 vs. 25/51, p = 0.36). Patient 11 with p.L168P in *HNF1B* had end-stage renal failure and was treated with peritoneal dialysis, while the remaining 11 carriers had no apparent extra-pancreatic complications.

We then compared the clinical characteristics between patients with *INS* mutations (n = 7) and those with *HNF* mutations (n = 5) (Table 4). Age at diagnosis was significantly lower in *INS* mutation carriers than that of *HNF* mutation carriers (2.3 vs. 10.2 years, p = 0.01). DKA was observed in two *INS* mutation carriers, but was not described in the *HNF* mutation carriers. Other examined parameters were comparable between the two groups.

DISCUSSION

NGS-based mutation screening identified probable pathogenic mutations in 11 of 89 patients with autoantibody-negative T1D. Identified mutations consisted of six substitutions in *INS*, two in *HNF1A*, two in *HNF4A*, and one in *HNF1B*. These results provide further evidence that monogenic mutations account for a small fraction of children with autoantibody-negative T1D. Since MLPA excluded copy-number variations of *GCK, HNF1A, HNF4A,* and *HNF1B* in our patients, chromosomal deletions involving these genes appear to be uncommon in this condition. Notably, the results of this study have both similarities and differences to those of the previous mutation screening by Johansson et al (8). Both studies demonstrated significant roles of *INS* and *HNF* genes, together with negligible roles of most other monogenic diabetes genes, in the development of autoantibody-negative T1D. However, the frequency of pathogenic *INS* mutations was much higher in our cohort than that in the cohort of Johansson et al. (6/89 vs. 1/469). In contrast, none of our participants carried *GCK* mutations which accounted for 6 of 469 patients studied by Johansson et al. Heterozygous *GCK* mutations are known to represent one of the major causes of MODY (3, 31, 32). These discrepancies between previous studies and ours likely reflect the differences in the clinical severities of the participants. It is known that *INS* mutations represent the second common cause of permanent neonatal diabetes (3, 22) and often result in insulin-requiring diabetes in early childhood, while heterozygous *GCK* mutations typically lead to relatively mild diabetes that does not require insulin therapy. Since our study group consisted solely of children with insulin-requiring diabetes, this selection criterion likely contributed to the accumulation of *INS* mutations and the lack of *GCK* mutations.

Of the 11 mutation carriers, only two had affected parents. These data imply that *INS* and *HNF* mutations can be associated with *de novo* occurrence or incomplete penetrance. We confirmed that at least three of the 11 mutations, i.e., p.V42A, p.G75C

and p.R89C in *INS*, were *de novo*. Although a *de novo* substitution can be a functionally benign variant (33, 34), pathogenicity of these three *INS* mutations was supported by multiple *in silico* programs. In addition, mutations p.V42A and p.R89C have already been reported as causing diabetes in childhood (22, 25, 35). Recently, Stanik et al. revealed that *de novo* mutations in *GCK*, *HNF1A*, and *HNF4A* are more frequent in MODY cases than previously assumed (36). Our data, in conjunction with those of Stanik et al., suggest that the *de novo* occurrence of *INS* or *HNF* mutations is not rare. Since previous sequence analyses of *INS* and *HNF* have focused primarily on patients with a positive family history (15, 37), further studies are necessary to clarify the actual frequency of these mutations among patients with diabetes.

The frequencies of susceptible and protective HLA-DRB1 alleles were significantly different between the carrier and non-carrier groups. The frequencies of these alleles in the carrier group were close to those seen in the Japanese general population, whereas the non-carrier group was characterized by relatively high and low frequencies of susceptibility and protective alleles, respectively. These results imply that a substantial fraction of the non-carrier group can be ascribed to autoimmune abnormalities, although we cannot exclude the possibility that some other monogenic diabetes genes remain unidentified. This is consistent with the prior findings by Hameed et al. that a substantial percentage of patients who had no autoantibodies at diagnosis were found to be antibody-positive at retesting. Since previous studies have revealed the ethnic specificity of protective and high risk HLA alleles for T1D (1, 2, 17), the contribution of autoimmune abnormality to T1D in the non-carrier group may differ among ethnic groups. Hameed et al. suggested that repeated measurement of blood C-peptide values provides useful information about the clinical course of T1D; relatively preserved C-peptide levels during the follow-up period are often seen in persistent antibody negative cases. Thus, C-peptide values of our patients need to be carefully monitored, although the values at diagnosis were highly variable and did not reflect the

presence or absence of monogenic mutations.

Clinical examinations revealed three notable findings. First, the clinical features of mutation carriers were comparable to those of non-carriers, except for the median BMI SD scores at diagnosis that were low in the non-carrier group and normal in the carrier group. The normally preserved BMI at diagnosis in the carrier group may reflect the slow progression of the disease, because mutations in *HNF1A, HNF4A*, and *HNF1B* are known to cause a gradual impairment of insulin secretion (38, 39). Second, patient 11 with a *HNF1B* mutation manifested renal failure. This is consistent with previous observations that renal cysts and renal dysplasia are common features of patients with *HNF1B* mutations (3). The presence of extra-pancreatic lesions appears to be a good marker of monogenic diabetes among patients with autoantibody-negative diabetes. Lastly, compared to carriers of *HNF* mutations, *INS* mutation carriers tended to have early disease onset and more frequently experienced DKA. These data are consistent with previous observations that *INS* mutations are associated with a both severe neonatal insulin deficiency and MODY, while *HNF* mutations typically lead to late-onset slowly progressive diabetes (33, 38, 39, 40). However, given the small number of participants in this study, further studies are necessary to clarify the frequency and phenotypic characteristics of each monogenic abnormality among autoantibody-negative T1D cases.

In conclusion, this study provides further evidence for the significant genetic overlap between autoantibody-negative T1D and monogenic diabetes. Mutations in *INS* and *HNF* genes, but not those in *GCK* and other monogenic diabetes genes, likely play critical roles in childhood-onset insulin-requiring T1D. This study also suggests the relatively high *de novo* rates of *INS* and *HNF* mutations, as well as the etiological link between autoimmune abnormalities and T1D in the non-carrier group. Carriers of monogenic mutations show nonspecific phenotypes among all T1D cases, although they are more likely to have a normal BMI at diagnosis than non-carriers.

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ACKNOWLEDGEMENTS

This work was supported by grant–in–aid for Scientific Research from the Japan Society for the Promotion of Science, by grants from the Manpei Suzuki Diabetes Foundation, the Japan Diabetes Foundation, the Japan Agency for Medical Research and Development, the National Center for Child Health and Development, and the Takeda foundation.

REFERENCES

1. Craig ME, Jefferies C, Dabelea D, Balde N, Seth A, Donaghue KC. ISPAD Clinical Practice Consensus Guidelines 2014. Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2014; 15 Suppl 20:4–17.
2. Redondo MJ, Eisenbarth GS. Genetic control of autoimmunity in Type I diabetes and associated disorders. Diabetologia. 2002; 45:605–622.
3. Rubio-Cabezas O, Hattersley AT, Njolstad PR et al. ISPAD Clinical Practice Consensus Guidelines 2014. The diagnosis and management of monogenic diabetes in children and adolescents. Pediatr Diabetes. 2014; 15 Suppl 20:47–64.
4. Slingerland AS. Monogenic diabetes in children and young adults: Challenges for researcher, clinician and patient. Rev Endocr Metab Disord. 2006; 7:171–185.
5. Kerns SL, Guevara-Aguirre J, Andrew S et al. A novel variant in CDKN1C is associated with intrauterine growth restriction, short stature, and early-adulthood-onset diabetes. J Clin Endocrinol Metab. 2014; 99:E2117–2122.
6. Biason-Lauber A, Boni-Schnetzler M, Hubbard BP et al. Identification of a SIRT1 mutation in a family with type 1 diabetes. Cell Metab. 2013; 17:448–455.
7. Cliffe ST, Kramer JM, Hussain K et al. SLC29A3 gene is mutated in pigmented hypertrichosis with insulin-dependent diabetes mellitus syndrome and interacts with the insulin signaling pathway. Hum Mol Genet. 2009; 18:2257–2265.
8. Johansson BB, Irgens HU, Molnes J et al. Targeted next-generation sequencing reveals MODY in up to 6.5% of antibody-negative diabetes cases listed in the Norwegian Childhood Diabetes Registry. Diabetologia. 2016 Dec 02.
9. Brahm AJ, Wang G, Wang J et al. Genetic Confirmation Rate in Clinically Suspected Maturity-Onset Diabetes of the Young. Can J Diabetes. 2016; 40:555–560.
10. Shepherd M, Shields B, Hammersley S et al. Systematic Population Screening, Using Biomarkers and Genetic Testing, Identifies 2.5% of the U.K. Pediatric

362 Diabetes Population With Monogenic Diabetes. Diabetes Care. 2016; 39:1879-
363 1888.
364 11. Rubio-Cabezas O, Edghill EL, Argente J, Hattersley AT. Testing for monogenic
365 diabetes among children and adolescents with antibody-negative clinically defined
366 Type 1 diabetes. Diabet Med. 2009; 26:1070–1074.
367 12. Hameed S, Ellard S, Woodhead HJ et al. Persistently autoantibody negative (PAN)
368 type 1 diabetes mellitus in children. Pediatr Diabetes. 2011; 12:142–149.
369 13. Moritani M, Yokota I, Tsubouchi K et al. Identification of INS and KCNJ11 gene
370 mutations in type 1B diabetes in Japanese children with onset of diabetes before 5
371 years of age. Pediatr Diabetes. 2013; 14:112–120.
372 14. Moritani M, Yokota I, Horikawa R et al. Identification of monogenic gene
373 mutations in Japanese subjects diagnosed with type 1B diabetes between >5 and
374 15.1 years of age. J Pediatr Endocrinol Metab. 2016; 29:1047–1054.
375 15. Yorifuji T, Fujimaru R, Hosokawa Y et al. Comprehensive molecular analysis of
376 Japanese patients with pediatric-onset MODY-type diabetes mellitus. Pediatr
377 Diabetes. 2012; 13:26–32.
378 16. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes
379 mellitus and its complications. Part 1: diagnosis and classification of diabetes
380 mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15:539–553.
381 17. Sugihara S, Ogata T, Kawamura T et al. HLA-class II and class I genotypes among
382 Japanese children with Type 1A diabetes and their families. Pediatr Diabetes. 2012;
383 13:33–44.
384 18. Kato N, Takimoto H, Sudo N. The Cubic Functions for Spline Smoothed L, S and
385 M Values for BMI Reference Data of Japanese Children. Clin Pediatr Endocrinol.
386 2011; 20:47–49.
387 19. Kawasaki E, Maruyama T, Imagawa A et al. Diagnostic criteria for acute-onset type
388 1 diabetes mellitus (2012): Report of the Committee of Japan Diabetes Society on

the Research of Fulminant and Acute-onset Type 1 Diabetes Mellitus. J Diabetes Investig. 2014; 5:115–118.

20. Wolfsdorf JI, Allgrove J, Craig ME et al. ISPAD Clinical Practice Consensus Guidelines 2014. Diabetic ketoacidosis and hyperglycemic hyperosmolar state. Pediatr Diabetes. 2014; 15 Suppl 20:154–179.
21. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013; 48:452–458.
22. Edghill EL, Flanagan SE, Patch AM et al. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes. 2008; 57:1034–1042.
23. Yamagata K, Oda N, Kaisaki PJ et al. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature. 1996; 384:455–458.
24. Colclough K, Bellanne-Chantelot C, Saint-Martin C, Flanagan SE, Ellard S. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha and 4 alpha in maturity-onset diabetes of the young and hyperinsulinemic hypoglycemia. Hum Mutat. 2013; 34:669–685.
25. Piccini B. Artuso R. Lenzi L et al. Clinical and molecular characterization of a novel INS mutation identified in patients with MODY phenotype. Eur J Med Genet. 2016; 59:590–595.
26. Liu M, Sun J, Cui J et al. INS-gene mutations: from genetics and beta cell biology to clinical disease. Mol Aspects Med. 2015; 42:3–18.
27. Wu G, Bohn S, Ryffel GU. The HNF1beta transcription factor has several domains involved in nephrogenesis and partially rescues Pax8/lim1-induced kidney malformations. Eur J Biochem. 2004; 271:3715–3728.
28. Lu P, Rha GB, Chi YI. Structural basis of disease-causing mutations in hepatocyte

nuclear factor 1beta. Biochemistry. 2007; 46:12071–12080.

29. Lu P, Rha GB, Melikishvili M et al. Structural basis of natural promoter recognition by a unique nuclear receptor, HNF4alpha. Diabetes gene product. J Biol Chem. 2008; 283:33685–33697.

30. Chandra V, Huang P, Potluri N, Wu D, Kim Y, Rastinejad F. Multidomain integration in the structure of the HNF-4alpha nuclear receptor complex. Nature. 2013; 495:394–398.

31. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med. 2001; 345:971–980.

32. Kawakita R, Hosokawa Y, Fujimaru R et al. Molecular and clinical characterization of glucokinase maturity-onset diabetes of the young (GCK-MODY) in Japanese patients. Diabet Med. 2014; 31:1357–1362.

33. Bonfanti R, Colombo C, Nocerino V et al. Insulin gene mutations as cause of diabetes in children negative for five type 1 diabetes autoantibodies. Diabetes Care. 2009; 32:123–125.

34. Liu M, Lara-Lemus R, Shan SO et al. Impaired cleavage of preproinsulin signal peptide linked to autosomal-dominant diabetes. Diabetes. 2012; 61:828–837.

35. Colombo C, Porzio O, Liu M et al. Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. J Clin Invest. 2008; 118:2148–2156.

36. Stanik J, Dusatkova P, Cinek O et al. De novo mutations of GCK, HNF1A and HNF4A may be more frequent in MODY than previously assumed. Diabetologia. 2014; 57:480–484.

37. Irgens HU, Molnes J, Johansson BB et al. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. Diabetologia. 2013; 56:1512–1519.

38. Amed S, Oram R. Maturity-Onset Diabetes of the Young (MODY): Making the Right Diagnosis to Optimize Treatment. Can J diabetes. 2016; 40:449–454.
39. Timsit J, Saint-Martin C, Dubois-Laforgue D, Bellanne-Chantelot C. Searching for Maturity-Onset Diabetes of the Young (MODY): When and What for? Can J diabetes. 2016; 40:455–461.
40. Molven A, Ringdal M, Nordbo AM et al. Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. Diabetes. 2008; 57:1131–1135.

Table 1. Characteristics of participants.

Total number	89
Male : Female	34 : 55
Age at diagnosis (yr)	8.2 (3.6–11.0)
Diabetes duration (yr)	3.3 (1.7–7.2)
Parental history of diabetes	8 / 75
BMI SD score at diagnosis	-1.0 ± 1.2
HbA1c at diagnosis (NGSP, %)	11.3 (9.5–13.4)
HbA1c at diagnosis (IFCC, mmol/mol)	99.6 (80.3–122.9)
C-peptide negative at diagnosis[#]	26 / 56
DKA at diagnosis*	24 / 69
Birth weight (g)	3,056 ± 404.0
Gestational age (weeks)	39.3 ± 1.5
Susceptible HLA-DRB1 alleles†	100 / 178
Protective HLA-DRB1 alleles†	13 / 178

BMI, Body Mass Index; DKA, diabetic ketoacidosis; IFCC, International Federation of Clinical Chemistry; NGSP, National Glycohemoglobin Standardization Program. Data are represented as median (interquartile range) or mean ± SD.

[#] Patients with a fasting C-peptide <0.6 ng/ml were assessed as C-peptide negative (19)

* DKA was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 (20).

† Susceptible and protective HLA-DRB1 alleles are defined based on the report of Sugihara et al (17).

Table 2. Clinical and molecular findings of mutation carriers.

Patient	Background		Clinical features at diagnosis						Monogenic substitutions		HLA-DRB1 alleles‡
	Gender	Affected parent*	Age (yr.)	BMI (SDS)	HbA1c (NGSP) (%)	DKA†	Autoantibodies tested at diagnosis	C-peptide (ng/mL)	Mutation	Parental origin	
1	M	father	4.9	0.1	7.9	no	GADA, IA2A	1.1	*INS* (p.C31Y)	father	***08:03***11:01
2	M	0	9.2	-1.0	10.0	no	GADA, IA2A	0.7	*INS* (p.V42A)	de novo	<u>*04:05*09:01</u>
3	F	0	3.1	-0.9	12.5	no	GADA, IA2A	no data	*INS* (p.G75C)	de novo	*04:10*13:02
4	M	0	0.7	-0.6	9.5	yes	GADA	no data	*INS* (p.R89C)	de novo	<u>*04:05</u>***15:02**
5	F	mother	2.3	0.1	9.7	no data	GADA, IA2A	no data	*INS* (p.C96F)	mother	*12:02***15:01**
brother of patient 5	M	mother	1.4	0.4	7.4	no data	GADA, IA2A	no data	*INS* (p.C96F)	mother	*12:01***15:01**
6	M	0	1.7	0.3	15.5	yes	GADA, IA2A	0.1	*INS* (p.C96R)	no data	*13:02***15:01**
7	F	0	10.2	-0.3	9.5	no data	GADA, IA2A	no data	*HNF1A* (p.R131Q)	no data	<u>*04:05</u>***15:01**
8	F	0	8.1	0.2	9.5	no data	GADA, IA2A	no data	*HNF1A* (p.R203S)	no data	*13:02*14:03
9	F	0	11.7	0.0	10.3	no	GADA, IA2A	3.1	*HNF4A* (p.Q142H)	no data	*04:03*14:54
10	F	0	9.2	1.7	10.5	no data	GADA, IA2A IAA	no data	*HNF4A* (p.E256A)	no data	*01:01*01:01
11	M	0	10.9	0.0	8.9	no	GADA	4.7	*HNF1B* (p.L168P)	no data	*04:03*13:02

BMI, Body Mass Index; DKA, diabetic ketoacidosis; F, female; GADA, glutamic acid decarboxylase 65 antibody; IA2A tyrosine phosphatase-like insulinoma antigen 2 antibody; IAA, insulin autoantibody; M, male; NGSP, National Glycohemoglobin Standardization Program.

* The number of parents with history of diabetes.

† DKA was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 (20).

‡ Susceptible HLA-DRB1 alleles are underlined, and protective alleles are boldfaced. These alleles were defined based on the report of Sugihara et al (17).

Variants are described according to the HGMD reference sequences: *HNF1A* NM_000545.6; *HNF4A* NM_175914.4; *HNF1B* NM_000458.3; *INS* NM_001185098.1.

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Table 3. Comparison between mutation carriers and non-carriers.

	Mutation carriers (n = 12)	Non-carriers (n = 78)	p-value
Male : Female	6 : 6	29 : 49	0.53
Age at diagnosis (yr)	6.5 (2.1–9.5)	8.3 (3.8–11.5)	0.12
Diabetes duration (yr)	2.9 (1.4–3.1)	3.7 (1.8–8.1)	0.14
Parental history of diabetes	3 / 12	6 / 64	0.15
BMI SD score at diagnosis	0 ± 0.7	-0.8 ± 1.2	0.02
HbA1c at diagnosis (NGSP, %)	9.6 (9.4–10.4)	11.7 (9.6–13.4)	0.08
C-peptide negative at diagnosis[#]	1 / 5	25 / 51	0.36
DKA at diagnosis[*]	2 / 7	22 / 62	1.00
Birth weight (g)	3,027 ± 404.5	3,058 ± 409.9	0.82
Gestational age (weeks)	38.8 ± 1.6	39.3 ± 1.5	0.33
Susceptible HLA-DRB1 alleles[†]	4 / 24	96 / 156	0.00004
Protective HLA-DRB1 alleles[†]	6 / 24	8 / 156	0.004

BMI, Body Mass Index; DKA, diabetic ketoacidosis; NGSP, National Glycohemoglobin Standardization Program. Data are represented as median (interquartile range) or mean ± SD.

[#] Patients with a fasting C-peptide <0.6 ng/ml were assessed as C-peptide negative (19).

[*] DKA was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 (20).

[†] Susceptible and protective HLA-DRB1 alleles are defined based on the report of Sugihara et al (17).

Table 4. Comparison between *INS* mutation carriers and carriers of *HNF1A*, *HNF4A*, and *HNF1B* mutations.

	INS mutation carriers (*n* = 7)	*HNF* mutation carriers (*n* = 5)	*p*-value
Male : Female	5 : 2	1 : 4	0.24
Age at diagnosis (yr)	2.3 (1.5–4.0)	10.2 (9.2–10.9)	0.01
Diabetes duration (yr)	3.0 (1.5–5.2)	2.9 (0.7–2.9)	0.34
Parental history of diabetes	3 / 7	0 / 5	0.21
DKA at diagnosis*	2 / 5	0 / 2	1.00
BMI SD score at diagnosis	-0.2 ± 0.6	0.32 ± 0.8	0.20
HbA1c at diagnosis (NGSP, %)	9.7 (8.7–11.3)	9.5 (9.5–10.3)	1.00
Birth weight (g)	3,045 ± 361.0	2,995 ± 531.7	0.86

BMI, Body Mass Index; DKA, diabetic ketoacidosis; NGSP, National Glycohemoglobin Standardization Program. Data are represented as median (interquartile range) or mean ± SD.

* DKA was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 (20).

FIGURE LEGENDS

Figure 1. Monogenic mutations identified in the present study. Chromatographs of patients 1–11 are shown. Arrows indicate mutated nucleotides. The S symbols depict disulfide bonds. DNA binding domains of *HNF1A* and *HNF1B* consist of the POU specific domain (POU_S) and the POU homeodomain (POU_H).

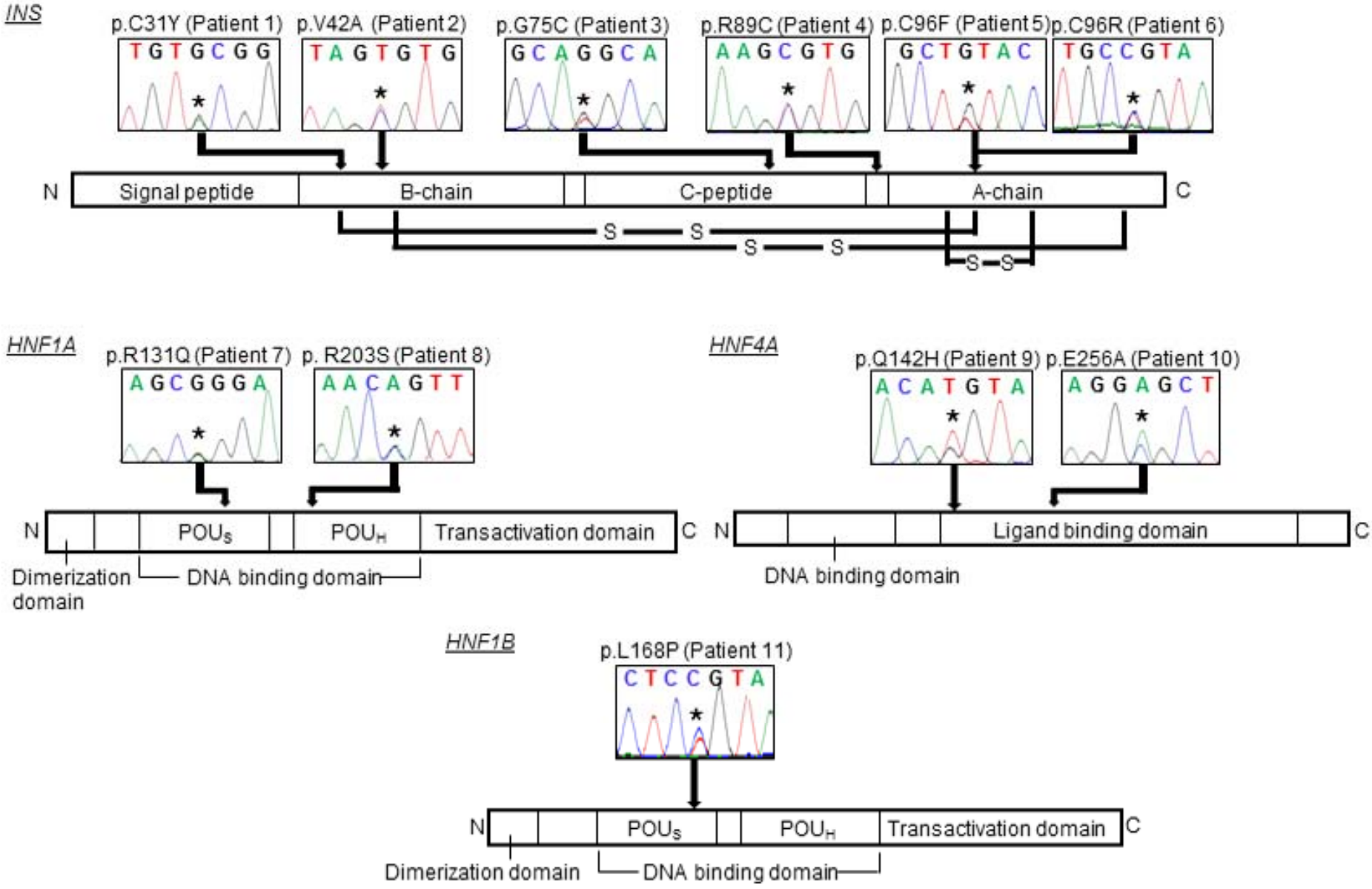
INS
p.C31Y (Patient 1)
p.V42A (Patient 2)
p.G75C (Patient 3)
p.R89C (Patient 4)
p.C96F (Patient 5)
p.C96R (Patient 6)
T G T G C G G
T A G T G T G
G C A G G C A
A A G C G T G
G C T G T A C
T G C C G T A
N
Signal peptide
B-chain
C-peptide
A-chain
C
S S
S S
S S
HNF1A
p.R131Q (Patient 7)
p. R203S (Patient 8)
A G C G G G A
A A C A G T T
N
POU$_S$
POU$_H$
Transactivation domain
C
Dimerization domain
DNA binding domain
HNF4A
p.Q142H (Patient 9)
p.E256A (Patient 10)
A C A T G T A
A G G A G C T
N
Ligand binding domain
C
DNA binding domain
HNF1B
p.L168P (Patient 11)
C T C C G T A
N
POU$_S$
POU$_H$
Transactivation domain
C
Dimerization domain
DNA binding domain

Table S1. Clinical findings of mutation non-carriers.

Patient	Background		Clinical findings at diagnosis						HLA-DRB1 alleles[‡]	
	Gender	Affected parent* (*n*)	Age (yr.)	BMI Z (SDS)	HbA1c (NGSP, %)	DKA[†]	Tested autoantibody	C-peptide (ng/mL)		
12	F	0	3.2	0.2	8.0	no data	GADA	no data	*04:05	*09:01
13	F	0	11.0	-2.3	14.8	yes	GADA, IA2A, IAA	0.1	*09:01	*09:01
14	F	0	13.3	-1.6	9.8	yes	GADA, IA2A	0.5	*04:04	*04:05
15	F	0	6.4	-1.5	15.7	no	GADA, IA2A	0.4	*09:01	*13:02
16	F	0	6.4	-1.8	13.7	yes	GADA	no data	*04:05	***08:03**
17	M	no data	13.0	-1.5	12.0	no data	GADA	0.4	*01:01	*04:05
18	F	no data	12.5	-0.8	10.2	no	GADA	1.5	*04:05	*13:02
19	F	0	13.6	0.3	15.3	yes	GADA, IA2A, IAA	0.6	*04:05	*13:02
20	M	0	15.1	-1.1	6.5	no	GADA, IA2A, IAA	0.3	*04:05	*04:05
21	M	no data	5.6	-1.4	15.3	no	GADA, IAA	< 0.3	*04:05	*04:05
22	M	no data	5.6	-1.1	8.9	no	GADA	0.8	*04:05	*08:02
23	F	0	10.1	1.4	9.8	no data	GADA, IA2A, IAA	no data	*09:01	*13:02
24	M	0	9.7	-1.3	11.3	no	GADA, IA2A, IAA	0.9	*04:05	*04:07
25	M	0	11.0	-1.2	11.3	no	GADA, IA2A, IAA	0.3	*04:05	*04:05
26	F	0	13.0	-1.2	10.2	no	GADA, IA2A, IAA	1.1	*04:05	*09:01
27	M	0	13.6	-1.5	12.1	no	GADA, IAA	1.5	*04:05	*13:02
28	M	0	8.1	-0.9	6.3	yes	GADA, IA2A, IAA	0.1	*04:05	*16:02
29	M	0	7.6	-1.0	11.1	no	GADA, IA2A	no data	*01:01	*04:05
30	M	1	4.1	-0.3	8.6	no data	GADA, IA2A, IAA	no data	*04:05	*04:07

31	F	0	2.5	-2.7	13.4	no data	GADA, IAA	no data	*09:01	*09:01
32	F	0	12.2	-2.1	15.6	no	GADA, IAA	no data	*01:01	*04:05
33	M	0	3.6	0.9	7.6	no	GADA	no data	*01:01	*09:01
34	F	0	2.3	-1.7	10.0	yes	GADA, IA2A	no data	*09:01	*09:01
35	M	1	10.7	1.1	11.7	no	GADA, IA2A	no data	*09:01	*13:02
36	F	0	6.1	-2.4	9.5	no	GADA, IAA	no data	*01:01	*04:05
37	F	0	4.9	-0.2	14.5	yes	GADA, IA2A	0.5	*09:01	*12:01
38	M	0	12.1	-1.3	5.6	yes	GADA	no data	*01:01	*09:01
39	F	1	8.6	2.5	12.9	no data	GADA, IAA	< 0.3	*04:03	***15:02**
40	F	0	14.4	-0.9	5.6	yes	GADA	0.1	*04:05	*08:02
41	F	no data	2.9	-3.1	12.8	yes	GADA, IA2A	no data	*04:05	*08:02
42	F	0	2.4	-1.6	14.7	no	GADA	< 0.3	*04:05	*13:02
43	F	0	4.3	0.5	13.2	yes	GADA, IAA	0.1	*04:05	*13:02
44	F	no data	11.5	-1.0	13.0	yes	GADA	no data	*04:05	*04:07
45	M	0	3.4	-3.1	12.3	no	GADA, IAA	0.1	*04:05	*08:02
46	F	no data	10.3	-2.1	13.3	yes	GADA	0.4	*09:01	*13:02
47	M	0	2.6	0.8	9.6	no	GADA	0.4	*01:01	*04:05
48	F	0	10.5	-0.9	10.4	no	GADA, IA2A	0.7	*04:10	*09:01
49	M	0	2.6	-0.3	12.3	no	GADA	0.1	*04:05	*13:02
50	F	0	5.3	0.6	6.2	no data	GADA, IA2A	0.7	*04:05	*11:01
51	F	0	9.0	-2.1	15.0	yes	GADA, IA2A, IAA	0.1	*04:05	*13:02
52	F	0	9.0	-2.1	11.2	no	GADA, IA2A, IAA	0.6	*09:01	*14:06
53	F	0	5.3	-0.6	8.7	no data	GADA	no data	*09:01	*13:02
54	M	0	2.6	-2.2	11.0	no data	GADA, ICA	no data	*04:05	*09:01
55	M	0	2.7	-0.4	13.4	yes	GADA, IA2A	no data	*09:01	*11:05
56	F	0	3.8	-2.3	13.2	no	GADA	0.5	*04:05	*09:01
57	M	0	1.1	0.6	11.5	no data	GADA	0.7	*04:05	*09:01

58	F	0	1.5	0.0	9.8	yes	GADA, IA2A, ICA	no data	*09:01	*13:02
59	F	0	4.9	0.0	11.9	no	GADA	0.5	*04:05	*12:01
60	M	0	10.7	-0.7	8.3	no	GADA, IA2A	1.1	*09:01	*09:01
61	M	0	11.8	-0.1	11.9	no	GADA	0.8	*01:01	*04:05
62	F	0	13.1	-0.7	12.4	no data	GADA, IA2A, ICA	0.9	*09:01	***15:01**
63	M	0	3.5	-0.4	11.3	no	GADA, IA2A, ICA	0.3	*04:05	*04:07
64	M	0	3.8	-0.9	14.5	no	GADA	0.6	*09:01	*13:02
65	M	0	14.7	-2.3	15.6	no data	GADA, IA2A	1.3	*08:02	*12:01
66	F	0	10.1	0.0	13.6	no	GADA, IA2A	1.2	*01:01	*09:01
67	F	0	7.5	-1.4	10.4	no	GADA, IA2A	0.8	*01:01	*04:05
68	F	1	1.7	-0.9	9.3	no data	GADA	0.1	*04:05	*08:02
69	F	1	3.6	0.1	11.7	yes	GADA	0.9	*09:01	*09:01
70	F	0	14.2	-1.4	9.6	yes	GADA, IA2A	1.0	*04:05	*11:01
71	F	0	3.4	-1.5	9.1	no	GADA	no data	*09:01	*13:02
72	M	0	0.9	0.0	6.8	no	GADA, IA2A	no data	*01:01	*04:05
73	F	0	5.2	-2.8	16.6	yes	GADA, IA2A	no data	*04:05	*09:01
74	F	0	8.6	0.4	13.6	no	GADA	0.8	*04:05	*13:02
75	F	0	8.4	0.9	5.8	no	GADA	2.2	*03:01	*04:05
76	F	0	13.3	1.2	13.4	yes	GADA, ICA	no data	*11:01	***15:01**
77	F	no data	15.1	-0.3	9.9	no	GADA, IA2A	1.2	*14:06	***15:02**
78	F	1	9.0	-0.7	14.8	no data	GADA	no data	*09:01	*13:02
79	M	no data	10.9	-0.9	7.1	no data	GADA	no data	*13:02	*13:02
80	M	no data	13.3	1.2	11.2	no data	GADA	no data	*09:01	*13:02
81	M	0	8.2	-1.1	15.7	yes	GADA, IA2A	0.2	*04:05	*08:02

82	F	0	11.5	-1.8	6.6	no	GADA, IA2A, ICA	3.6	*04:05	*09:01
83	F	no data	5.5	-2.6	17.0	yes	GADA	0.2	*04:05	*08:02
84	F	0	14.0	1.2	14.5	no	GADA	1.0	*04:05	*13:02
85	F	no data	11.5	-1.2	14.9	no	GADA, ICA	1.2	***08:03**	***08:03**
86	M	no data	10.6	-1.6	6.9	no	GADA, IA2A	0.7	*04:05	*09:01
87	F	no data	2.4	-0.1	13.0	no	GADA	no data	*09:01	*13:02
88	F	0	10.3	0.9	12.0	no	GADA, IA2A	no data	*09:01	***15:02**
89	F	0	4.3	-1.7	12.4	no	GADA, IA2A, IAA	0.3	*09:01	*16:02

BMI, Body Mass Index; DKA, diabetic ketoacidosis; F, female; GADA, glutamic acid decarboxylase 65 antibody; IA2A, tyrosine phosphatase-like insulinoma antigen 2 antibody; IAA, insulin autoantibody; ICA, islet cell antibody; M, male; NGSP, National Glycohemoglobin Standardization Program.

Patients 1-11 had monogenic mutations (see Table 2).

* The number of parents with history of diabetes.

† DKA was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 (20).

‡ Susceptible HLA-DRB1 alleles are underlined, and protective alleles are boldfaced. These alleles were defined based on the report of Sugihara et al (17).

Variants are described according to the HGMD reference sequences: *HNF1A* NM_000545.6; *HNF4A* NM_175914.4; *HNF1B* NM_000458.3; *INS* NM_001185098.1.

Table S2. Mutations identified in present study.

Patient	Gene	Mutation		Population frequency in ExAC	Functional prediction					Previously identified in patients
		Protein change	Nucleotide change		SIFT	PolyPhen-2	Mutation Taster	CADD	M-CAP	
1	*INS*	p.C31Y	c.92G>A	no data	**0.000**	**1.000**	**disease causing**	**27.3**	**0.917**	yes[13)]
2	*INS*	p.V42A	c.125T>C	no data	**0.006**	**0.891**	**disease causing**	**24.5**	**0.708**	yes[25)]
3	*INS*	p.G75C	c.223G>T	no data	**0.010**	**1.000**	polymorphism	**22.8**	**0.228**	no
4	*INS*	p.R89C	c.265C>T	no data	**0.000**	**1.000**	**disease causing**	**26.3**	**0.800**	yes[22)]
5	*INS*	p.C96F	c.287G>T	no data	**0.001**	**1.000**	**disease causing**	**27.9**	**0.912**	no
6	*INS*	p.C96R	c.286T>C	no data	**0.001**	**1.000**	**disease causing**	**24.7**	**0.938**	yes[13)]
7	*HNF1A*	p.R131Q	c.392G>A	1 / 121,198	**0.002**	**1.000**	**disease causing**	**34.0**	**0.925**	yes[23)]
8	*HNF1A*	p.R203S	c.607C>A	no data	**0.001**	**0.995**	**disease causing**	**26.7**	**0.880**	yes[24)]
9	*HNF4A*	p.Q142H	c.426G>T	no data	0.121	0.000	**disease causing**	**21.1**	no data	no
10	*HNF4A*	p.E256A	c.767A>C	1 / 121,396	**0.002**	0.004	**disease causing**	**25.2**	**0.467**	no
11	*HNF1B*	p.L168P	c.503T>C	no data	**0.000**	**1.000**	**disease causing**	**28.8**	**0.721**	no

CADD, the Combined Annotation Dependent Depletion; ExAC, the Exome Aggregation Consortium Browser; M-CAP, the Mendelian

Clinically Applicable Pathogenicity; SIFT, Sorting Intolerant From Tolerant.

Scores classified as pathogenic by *in silico* analysis (SIFT scores < 0.05; PolyPhen-2 scores > 0.8; CADD scores > 20; and M-CAP scores > 0.025) are boldfaced.