ORIGINAL ARTICLE

Clinical and genetic characteristics of abnormal glucose tolerance in Japanese women in the first year after gestational diabetes mellitus

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ABSTRACT

Aims/Introduction: Risk factors of type 2 diabetes mellitus in Japanese women with recent gestational diabetes mellitus are unknown. The objective of the present study was to investigate the clinical and genetic characteristics associated with postpartum abnormal glucose tolerance in Japanese women with gestational diabetes mellitus.

Materials and Methods: A total of 213 Japanese women with recent gestational diabetes mellitus who underwent a postpartum 2-h oral glucose tolerance test were investigated. The association between antepartum clinical characteristics and postpartum abnormal glucose tolerance (diabetes or prediabetes based on the Japan Diabetes Society criteria) was examined. Frequencies of 45 known type 2 diabetes mellitus-associated genetic variants were also compared between women with and without postpartum abnormal glucose tolerance.

Results: A total of 59 women showed postpartum abnormal glucose tolerance (prediabetes, $n = 51$; diabetes, $n = 8$). Plasma glucose levels at 1 or 2 h, the insulinogenic index and the insulin secretion-sensitivity index-2 of the antepartum oral glucose tolerance test were independent of postpartum abnormal glucose tolerance risk factors ($P = 0.006$, $P = 0.00002$, $P = 0.01$ and $P = 0.006$, respectively). Four genetic variants (rs266729 [*ADI-POQ*], rs6017317 [*HNF4A*], rs5215 [*KCNJ11*] and rs7177055 [*HMG20A*]) showed a nominally significant association with postpartum abnormal glucose tolerance ($P < 0.05$, respectively). Among these, three were related to insulin secretion. Postpartum abnormal glucose tolerance risk significantly increased with increasing risk-allele number ($P = 0.0005$; odds ratio 1.91).

Conclusions: Clinical features and genetic variants related to impaired insulin secretion are risk factors of postpartum abnormal glucose tolerance in Japanese women with recent gestational diabetes mellitus.

INTRODUCTION

Women with a history of gestational diabetes mellitus (GDM) are at a high risk of developing type 2 diabetes mellitus in the future; therefore, postpartum diabetes screening is highly recommended for the improvement of health outcomes[1–3]. However, the rate of postpartum follow up for women with GDM is low; therefore, it is desirable to identify those at the greatest risk by determining the risk factors[3]. The clinical risk factors associated with postpartum abnormal glucose tolerance (pAGT) include a family history of diabetes, obesity, early GDM diagnosis, fasting hyperglycemia, insulin requirement during pregnancy and antepartum β-cell dysfunction[4–13]. Additionally, recent studies have shown that certain genetic variants are associated with the development of future type 2 diabetes mellitus in women with a history of GDM[9,14,15]. It is shown that cyclin-

dependent kinase inhibitor 2A/2B (*CDKN2A/2B*), hematopoietically expressed homeobox (*HHEX*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), transcription factor 7-like 2 (*TCF7L2*), and fat mass and obesity-associated protein (*FTO*) genes could be candidates for risk variants of pAGT[9,14,15]. Given that the susceptibility to glucose intolerance varies among different racial or ethnic groups[16,17], the clinical risk factors and genetic variants of pAGT might differ between Caucasian and Japanese women. However, few reports on the risk factors of pAGT are available with regard to Japanese women with GDM[10–12], and, to the best of our knowledge, there are no data on the genetic variants associated with pAGT in this population subgroup.

These analyses are further complicated by a lack of international agreement regarding the diagnostic criteria of GDM. The diagnosis of GDM in most of the studies on maternal characteristics associated with postpartum glucose intolerance was based on the Carpenter–Coustan (i.e., a 100-g, 3-h oral glucose tolerance test [OGTT])[18] or the World Health Organization (WHO) 1999 (i.e., a 75-g, 2-h OGTT) criteria[19]. In 2010, the International Association of Diabetes in Pregnancy Study Group (IADPSG) proposed new criteria for the diagnosis of GDM[20]. Since 2013, the WHO has also advised the use of the IADPSG criteria for the diagnosis of GDM[21]; however, the risk factors of pAGT in GDM according to the IADPSG criteria (i.e., WHO 2013 criteria) are yet unknown, because the number of healthcare providers adopting the new criteria remains limited.

With this background, we retrospectively investigated the postpartum glucose tolerance status in Japanese women with recent GDM using the IADPSG criteria (i.e., WHO 2013 criteria). Furthermore, we analyzed antenatal clinical and genetic characteristics in Japanese women with recently developed GDM and those having pAGT. We also examined the association between previously reported type 2 diabetes mellitus- or GDM-susceptibility genes and the development of pAGT in Japanese women with recently developed GDM.

METHODS
Participants
We retrospectively investigated a cohort of 213 women with a recent history of GDM who underwent postpartum diabetes screening at Keio University Hospital or the National Center for Child Health and Development between April 2011 and December 2016. During the study period, GDM was diagnosed according to the IADPSG criteria proposed in 2010[20]. Women with multifetal pregnancies and women whose neonates showed congenital anomalies were excluded from the present study. Women with overt diabetes in pregnancy and pre-pregnancy diabetes were also excluded. The research was carried out in accordance with the Declaration of Helsinki, and informed consent was obtained from patients where appropriate. This study was approved by the Keio University School of Medicine Ethics Committee (Nos. 20100154 and 20110321) and the institutional review board of the National Research Institute for Child Health and Development (No. 406).

Postpartum glucose tolerance status
Each woman with GDM was scheduled to undergo a postpartum diabetes screening using the 75-g OGTT ~6–12 weeks after delivery as part of the routine care recommendation by the Japan Society of Obstetrics and Gynecology[22]. Postpartum diabetes screening was rearranged if the mother failed to attend the scheduled appointment. According to the results of the postpartum OGTT, women with recently developed GDM were characterized into three categories based on the Japan Diabetes Society criteria: diabetic, fasting plasma glucose (PG) $\geq$126 mg/dL (7.0 mmol/L) and/or 2-h PG $\geq$200 mg/dL (11.1 mmol/L); normal, fasting PG $\leq$110 mg/dL (6.1 mmol/L) and 2-h PG $\leq$140 mg/dL (7.8 mmol/L); or prediabetes, neither normal nor diabetic[23]. In the present study, pAGT included women classified as either the diabetic or prediabetes type. Postpartum normal glucose tolerance (NGT) was defined as postpartum normal OGTT results.

Antepartum clinical and metabolic characteristics
Maternal characteristics in the index pregnancy were collected from patient hospital records as follows: maternal age, pregravid body mass index (BMI), a first-degree family history of diabetes, gestational age at the time of diagnosis of GDM and insulin requirement in pregnancy. Participants were categorized by pregravid BMI: underweight, BMI <18.5; normal weight, BMI from 18.5–24.9; and overweight, BMI $\geq$25.0[22]. Glycemic and insulin profiles of the OGTT, as well as levels of glycated hemoglobin at the time of diagnosis of GDM, were also reviewed. Additionally, metabolic features (i.e., insulin sensitivity, insulin secretion and β-cell function) were assessed using antepartum OGTT results. Insulin sensitivity was estimated according to the whole-body insulin sensitivity index derived from the OGTT (IS_{OGTT}) and the homeostasis model assessment for insulin resistance. The IS_{OGTT} was calculated using the following formula: 10,000/square root {$PG_0 \times Ins_0 \times (PG_0 + PG_{60} \times 2 + PG_{120})/2 \times (Ins_0 + Ins_{60} \times 2 + Ins_{120})/2$}, where PG_y (mg/dL) and Ins_y (mU/L) represent PG and insulin values, respectively, at time y min during the OGTT[24]. Homeostasis model assessment for insulin resistance was calculated as follows: $Ins_0 \times PG_0/405$[25]. Insulin secretion was assessed according to the insulinogenic index (IGI: {$Ins_{30} - Ins_0$}/{$PG_{30} - PG_0$}) and the ratio of the total area under the insulin curve to the total area under the glucose curve ($AUC_{ins/glu}$) during the OGTT[26]. To evaluate β-cell function, we calculated the OGTT-derived disposition index using the Insulin Secretion-Sensitivity Index-2 (ISSI-2; the $AUC_{ins/glu}$ multiplied by IS_{OGTT})[27].

Single-nucleotide polymorphism selection and genotyping
Single-nucleotide polymorphism (SNP) selection from previously reported type 2 diabetes mellitus- or GDM-susceptibility

genes and genotyping were carried out as previously described[28]. We selected the SNPs based on the criterion of minor allele frequency (MAF) >30% in the Japanese population, because this selection could provide adequate statistical power to detect SNPs with genotype relative risk ≥1.6 in our study cohort. Finally, we confirmed the association between 45 SNPs from 36 genes and the development of pAGT. All the polymorphisms analyzed in the present study were in Hardy–Weinberg equilibrium.

During the study period, maternal peripheral blood samples were collected soon after delivery, and genomic deoxyribonucleic acid was extracted using the QIAsymphony DNA mini kit (96) (Qiagen, Valencia, CA, USA) for exploratory research when informed consent was obtained. As all of the 213 women agreed to the present study, genotyping was carried out using the high-throughput genotyping MassARRAY platform (Sequenom Inc., San Diego, CA, USA) after postpartum OGTT. Primers, including those used for amplification and extension, were designed using Assay Design Suite (Sequenom Inc.; https://seqpws1.sequenom.com/AssayDesignerSuite.html; Table S1). Negative controls, run at least in quadruplicate, were placed on all 384 plates as quality controls. The SNP genotyping success rate was >94%, and the concordance rate for genotyping was >99.8% in the present study.

Statistical analysis

Data are presented as the median (range), mean ± standard deviation or the number of cases (percentage). Continuous data were compared between groups using Student's t-test or logistic regression analysis. Categorical variables were analyzed by the χ^2-test or Fisher's exact test.

For metabolic measurements, multivariate regression analysis was used to identify independent risk factors for pAGT. Additionally, predictive values of clinical characteristics for the risk of pAGT were obtained using multiple logistic regression analysis and receiver operating characteristics (ROC) analysis. For genetic variants, we analyzed the association between 45 SNPs (36 genes) and the risk of pAGT among 213 women. Per-allele odds ratios (ORs) and their 95% confidence intervals (CIs) for the association between SNPs and pAGT were evaluated using logistic regression analysis adjusted for maternal age, pregravid BMI, family history of type 2 diabetes mellitus and antepartum metabolic features that were significantly associated with pAGT (i.e., 2-h PG). The possibility of multiple testing burden was avoided by Bonferroni correction, and an adjusted $P < 0.05$ was considered significant. Therefore, we examined the combined effects of multiple genetic variants on pAGT in Japanese women with recent GDM and the cumulative effects of risk alleles at pAGT-associated SNPs having a lower P-value. Statistical analyses, the calculation of linkage disequilibrium among SNPs, and construction of a forest plot of per-allele ORs were carried out using R (version 3.3.1; https://cran.r-project.org/bin/macosx/).

RESULTS

Antepartum clinical and metabolic characteristics in women with a recent history of GDM

Women in the present study cohort underwent postpartum OGTT at a median of 24.9 weeks (range 6.0–53.7 weeks). At the time of GDM diagnosis, 142 women had a single abnormal OGTT value, 51 had two abnormal values and 20 had three abnormal values. Maternal age at delivery and pregravid BMI in this study cohort were 37 years (range 23–51 years) and 21.6 (range 16.2–38.8 tears), respectively. Of all the women, 128 (60%) were nulliparous.

During the follow-up period, 59 women (28%) developed pAGT: eight had diabetes and 51 had prediabetes as classified according to the Japan Diabetes Society criteria. These included 33 of 142 (23.2%) women with a single abnormal antepartum OGTT value, 18 of 51 (35.3%) with two abnormal values and eight of 20 (40.0%) with three abnormal values.

There were no significant differences in pregravid BMI, overweight status, GDM diagnosed before 20 weeks of pregnancy, and insulin requirement during pregnancy between the pAGT and NGT groups (Table 1). Women with pAGT showed older maternal age and a higher rate of family history of diabetes as compared with those in the NGT group. With regard to antepartum OGTT profile, women with pAGT showed significantly higher levels of antepartum 1-h PG and 2-h PG, as compared with those in the NGT group ($P < 0.001$). Among antepartum metabolic features, IGI in the pAGT group was significantly lower than that in the NGT group ($P < 0.01$). There was a significant difference in antepartum ISSI-2 between the pAGT and NGT groups ($P < 0.001$). After adjustment for maternal age and family history of diabetes using the logistic regression model, 1-h PG, 2-h PG, IGI and ISSI-2 derived from the antenatal OGTT remained independent risk factors of pAGT ($P = 0.006$, $P = 0.00002$, $P = 0.01$ and $P = 0.006$, respectively). The AUC was used to evaluate the predictive power of these antepartum factors (Table 2). Among clinical characteristics, the 2-h PG showed the largest area under the ROC curve (AUC 0.72).

Genetic variants associated with pAGT in women with a recent history of GDM

We compared the risk-allele frequencies of 45 SNPs between the pAGT and NGT groups. The individual SNP results are shown in Figure 1 and Table 3. After adjustment for maternal age at delivery, pregravid BMI, family history of type 2 diabetes mellitus and antepartum 2-h PG, four out of the 45 SNPs showed a nominally significant association with the development of pAGT (rs266729 [$P = 0.029$, OR 2.02, 95% CI 1.08–3.78], rs6017317 [$P = 0.031$, OR 1.77, 95% CI 1.05–2.99], rs5215 [$P = 0.032$, OR 1.82, 95% CI 1.05–3.14] and rs7177055 [$P = 0.043$, OR 1.82, 95% CI 1.02–3.26]). Therefore, we carried out a combined analysis of the four identified risk alleles, for which each individual could harbor between zero and eight possible risk alleles. With the increasing number of risk alleles,

Table 1 | Comparison of antepartum clinical features between women with postpartum abnormal glucose tolerance and normal glucose tolerance

	Abnormal glucose tolerance ($n = 59$)	Normal glucose tolerance ($n = 154$)
Maternal age at delivery (years)	39 (27–51)	37 (23–46)*
Pregravid BMI (kg/m²)	21.4 (17.0–33.9)	21.6 (16.2–38.8)
Pregravid overweight	18 (31)	27 (17)
Nulliparous	41 (69)	87 (56)
Family history of diabetes	19 (32)	22 (14)**
GW at diagnosis of GDM (weeks)	21 (9–33)	16 (7–34)
GDM diagnosed before 20 weeks of pregnancy	28 (47)	84 (55)
Insulin use in pregnancy	29 (49)	54 (35)
Plasma glucose of the antepartum OGTT (mg/dL)		
0 min	88 (76–109)	92 (68–116)*
30 min	157 (107–211)	153 (79–208)
60 min	182 (110–242)	165 (88–272)***
120 min	164 (112–230)	147 (88–256)***
Insulinogenic index	0.56 (0.17–1.71)	0.69 (0.02–7.53)**
IS$_{OGTT}$	2.69 (0.74–6.91)	2.53 (0.54–7.27)
HOMA-IR	1.29 (0.39–6.57)	1.49 (0.38–6.84)
ISSI-2	0.80 (0.41–1.62)	0.94 (0.36–2.51)***
HbA1c at diagnosis of GDM (%)	5.4 (4.8–6.1)	5.3 (4.3–6.1)

Data are median (range) or n (%). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. BMI, body mass index; GDM, gestational diabetes mellitus; GW, gestational week; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment for insulin resistance; IS$_{OGTT}$, insulin sensitivity index from the oral glucose tolerance test; ISSI-2, Insulin Secretion-Sensitivity Index-2; OGTT, oral glucose tolerance test; overweight, body mass index ≥25 kg/m².

Table 2 | Predictive values of antepartum clinical characteristics for the risk of postpartum abnormal glucose tolerance

	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1-h PG	0.66	0.58–0.74	175	66.1	58.4	37.9	81.8
2-h PG	0.72	0.64–0.80	156	74.6	61.7	42.7	86.4
Insulinogenic Index	0.62	0.54–0.70	0.66	65.5	52.6	34.5	80.0
ISSI-2	0.65	0.57–0.73	0.88	70.7	56.6	38.3	83.5

1-h PG, plasma glucose at 1 h in the antepartum oral glucose-tolerance test; 2-h PG, plasma glucose at 2 h in the antepartum oral glucose-tolerance test; AUC, area under the receiver operating characteristics curve; CI, confidence interval; IGI, Insulinogenic Index; ISSI-2, Insulin Secretion-Sensitivity Index-2; NPV, negative predictive value; PPV, positive predictive value.

the risk of pAGT increased ($P = 0.00005$, OR 1.91, 95% CI 1.40–2.61; Figure 2a). In particular, women with seven or more risk alleles showed a 7.67-fold increased risk of pAGT ($P = 0.0085$, 95% CI 1.68–34.9), as compared with those having three or fewer risk alleles (Figure 2b). The linkage disequilibrium values among four SNPs calculated using r^2 were <0.02 in both the pAGT and NGT groups. Additionally, we carried out the association analysis between the number of identified risk alleles and postpartum metabolic features using linear regression analysis. The 1 h-PG, 2 h-PG and ISSI-2 from the postpartum OGTT were significantly associated with increasing the number of risk alleles ($P = 0.00027$, $P = 0.008$ and $P = 0.0087$, respectively; Table S2).

DISCUSSION

As women with GDM are at a high risk of progression to prediabetes or type 2 diabetes mellitus, risk factors associated with

the development of this condition have received special attention[8]. The majority of studies have focused on the clinical features of GDM mainly as defined by the Carpenter–Coustan[18] or WHO 1999 criteria[19]. In contrast to genetic studies on the development of GDM, reports on genetic risk variants associated with glucose intolerance after pregnancies with GDM are very limited. In particular, there is a paucity of information on the risk of pAGT in Japanese women with a history of GDM. To the best of our knowledge, this is the first report highlighting both clinical and genetic characteristics associated with pAGT in Japanese women with recently developed GDM, as defined by the IADPSG criteria (i.e., WHO 2013 criteria).

The rate of pAGT (i.e., diabetes and prediabetes, as defined by Japan Diabetes Society criteria) was 28% in the present study cohort. In comparison, O'Reilly et al.[29] reported that 19% of women with GDM defined by the IADPSG criteria showed abnormal glucose tolerance (impaired fasting glucose, impaired
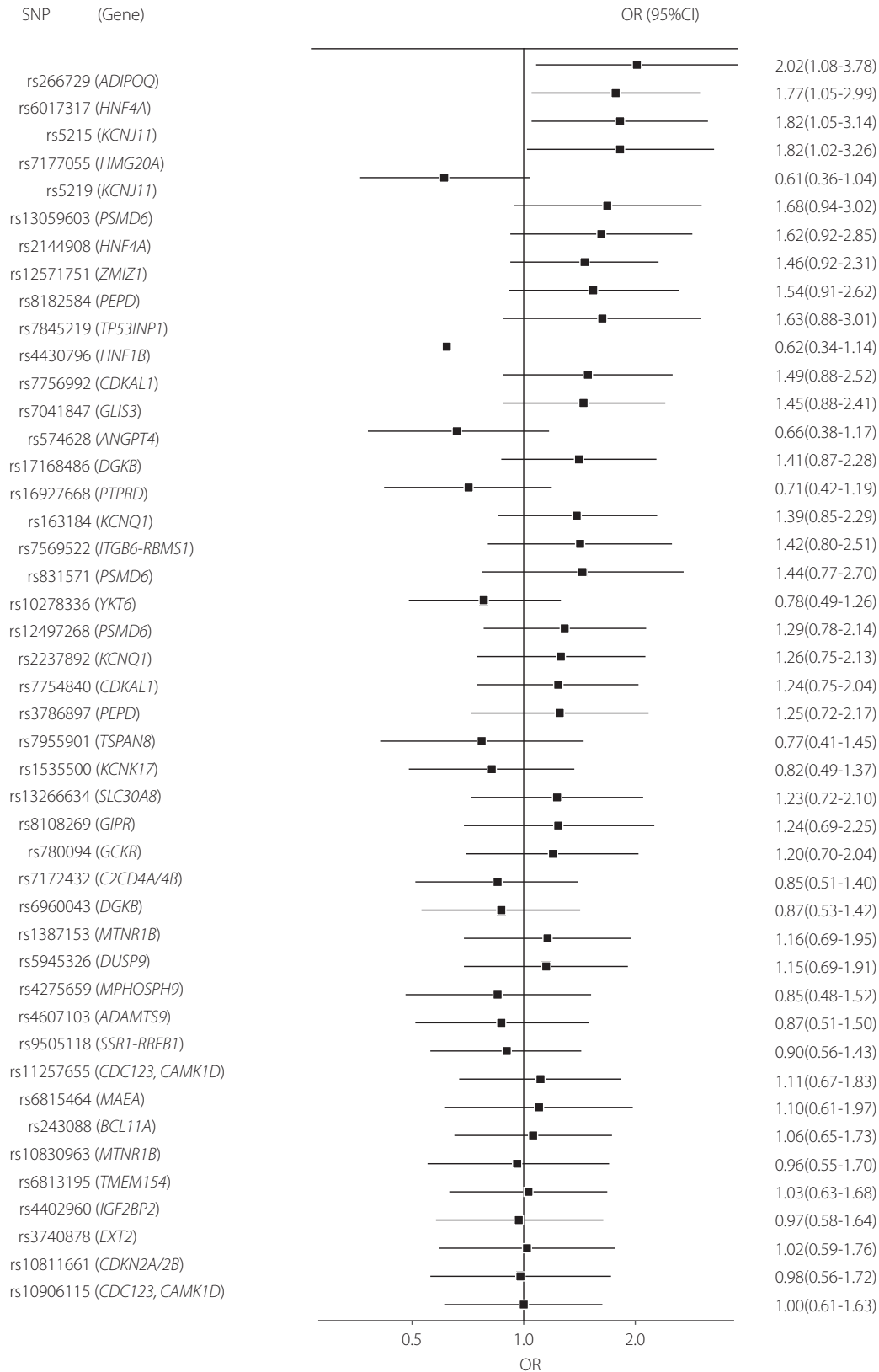
Figure 1 | Forest plot of per-allele odds ratio of 45 single-nucleotide polymorphisms assessed in the present study. CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 3 | Association analysis results between single nucleotide polymorphisms and the risk of gestational diabetes mellitus

	SNP	Chr	Nearby gene	Insulin sensitivity	Insulin secretion	Risk allele	Japanese major allele	Japanese minor allele	RAF of Japanese pAGT	The present study P-value	The present study OR (95% CI)
1	rs266729	3	ADIPOQ	Yes		C	C	G	0.69	0.029	2.02 (1.08–3.78)
2	rs6017317	20	HNF4A		Yes	G	G	T	0.55	0.031	1.77 (1.05–2.99)
3	rs5215	11	KCNJ11		Yes	T	T	C	0.34	0.032	1.82 (1.05–3.14)
4	rs7177055	15	HMG20A		Yes	A	G	A	0.41	0.043	1.82 (1.02–3.26)
5	rs5219	11	KCNJ11		Yes	T	C	T	0.35	0.07	0.61 (0.36–1.04)
6	rs13059603	3	PSMD6(THOC7)	Yes	Yes	A	A	G	0.68	0.08	1.68 (0.94–3.02)
7	rs2144908	20	HNF4A		Yes	A	G	A	0.42	0.09	1.62 (0.92–2.85)
8	rs12571751	10	ZMIZ1			G	A	G	0.42	0.11	1.46 (0.92–2.31)
9	rs8182584	19	PEPD	Yes		T	T	G	0.64	0.11	1.54 (0.91–2.62)
10	rs7845219	8	TP53INP1		Yes	T	C	T	0.27	0.12	1.63 (0.88–3.01)
11	rs4430796	17	HNF1B		Yes	G	A	G	0.36	0.13	0.62 (0.34–1.14)
12	rs7756992	6	CDKAL1		Yes	G	A	G	0.46	0.14	1.49 (0.88–2.52)
13	rs7041847	9	GLIS3		Yes	A	G	A	0.47	0.15	1.45 (0.88–2.41)
14	rs574628	20	ANGPT4	Yes		G	G	A	0.63	0.16	0.66 (0.38–1.17)
15	rs17168486	7	DGKB		Yes	T	C	T	0.41	0.17	1.41 (0.87–2.28)
16	rs16927668	9	PTPRD	Yes		T	T	C	0.57	0.19	0.71 (0.42–1.19)
17	rs163184	11	KCNQ1		Yes	G	T	G	0.42	0.19	1.39 (0.85–2.29)
18	rs7569522	2	ITGB6-RBMS1	Yes		A	G	A	0.38	0.23	1.42 (0.80–2.51)
19	rs831571	3	PSMD6(PRICKLE2)	Yes	Yes	C	C	T	0.69	0.26	1.44 (0.77–2.70)
20	rs10278336	7	YKT6			A	A	G	0.57	0.31	0.78 (0.49–1.26)
21	rs12497268	3	PSMD6(PRICKLE2)		Yes	G	G	C	0.61	0.32	1.29 (0.78–2.14)
22	rs2237892	11	KCNQ1		Yes	C	G	T	0.64	0.38	1.26 (0.75–2.13)
23	rs7754840	6	CDKAL1		Yes	C	G	C	0.41	0.40	1.24 (0.75–2.04)
24	rs3786897	19	PEPD	Yes		A	A	G	0.55	0.42	1.25 (0.72–2.17)
25	rs7955901	12	TSPAN8, LGR5		Yes	C	C	T	0.70	0.42	0.77 (0.41–1.45)
26	rs1535500	6	KCNK17			T	G	T	0.40	0.43	0.82 (0.49–1.37)
27	rs13266634	8	SLC30A8		Yes	C	C	T	0.56	0.44	1.23 (0.72–2.10)
28	rs8108269	19	GIPR	Yes		G	G	T	0.61	0.47	1.24 (0.69–2.25)
29	rs780094	2	GCKR	Yes		G	A	G	0.44	0.50	1.20 (0.70–2.04)
30	rs7172432	15	C2CD4A/4B	Yes	Yes	A	A	G	0.55	0.52	0.85 (0.51–1.40)
31	rs6960043	7	DGKB		Yes	C	C	T	0.50	0.58	0.87 (0.53–1.42)
32	rs1387153	11	MTNR1B		Yes	T	C	T	0.48	0.58	1.16 (0.69–1.95)
33	rs5945326	X	DUSP9	Yes		A	A	G	0.62	0.60	1.15 (0.69–1.91)
34	rs4275659	12	MPHOSPH9(ABCB9)			C	C	T	0.67	0.60	0.85 (0.48–1.52)
35	rs4607103	3	ADAMTS9	Yes		C	C	T	0.59	0.62	0.87 (0.51–1.50)
36	rs9505118	6	SSR1-RREB1		Yes	A	A	G	0.59	0.65	0.90 (0.56–1.43)
37	rs11257655	10	CDC123, CAMK1D		Yes	T	C	T	0.41	0.69	1.11 (0.67–1.83)
38	rs6815464	4	MAEA	Yes		C	C	G	0.64	0.76	1.10 (0.61–1.97)
39	rs243088	2	BCL11A	Yes		T	A	T	0.27	0.82	1.06 (0.65–1.73)
40	rs10830963	11	MTNR1B		Yes	G	C	G	0.47	0.90	0.96 (0.55–1.70)

Table 3 | (Continued)

SNP	Chr	Nearby gene	Insulin sensitivity	Insulin secretion	Risk allele	Japanese major allele	Japanese minor allele	RAF of Japanese pAGT	The present study P-value	OR (95% CI)
41 rs6813195	4	TMEM154		Yes	C	T	C	0.46	0.91	1.03 (0.63–1.68)
42 rs4402960	3	IGF2BP2		Yes	T	G	T	0.30	0.92	0.97 (0.58–1.64)
43 rs3740878	11	EXT2		Yes	A	A	G	0.64	0.95	1.02 (0.59–1.76)
44 rs10811661	9	CDKN2A/2B		Yes	T	T	C	0.52	0.96	0.98 (0.56–1.72)
45 rs10906115	10	CDC123, CAMK1D		Yes	A	A	G	0.51	0.99	1.00 (0.61–1.63)

P-values before Bonferroni correction are shown. Chr, chromosome; CI, confidence interval; OR, odds ratio; pAGT, postpartum abnormal glucose tolerance; RAF, risk allele frequency; SNP, single-nucleotide polymorphism.

glucose intolerance, both or type 2 diabetes mellitus using the American Diabetes Association criteria) in the tests carried out up to 6 months postpartum. Retnakaran et al.[1] showed that ~25% of women with gestational glucose intolerance (i.e., a single abnormal OGTT value and/or GDM by the Carpenter–Coustan criteria) developed prediabetes or diabetes as defined by the WHO 1999 criteria within the first year postpartum. Notably, gestational glucose intolerance defined in the study of Retnakaran et al.[1] was comparable with GDM according to the IADPSG criteria. These findings suggested that women with recently developed GDM, as defined by the IADPSG criteria, are at risk of pAGT, although the IADPSG diagnostic threshold was based on the risk for adverse perinatal outcomes associated with hyperglycemia. Those with a single abnormal value in the OGTT are thought to have less severe dysglycemia as compared with women with two or three abnormal values. However, the present results highlighted that even women with a single abnormal value have a ~25% risk of glucose intolerance after delivery, which should be recognized by clinicians.

Risk factors of pAGT might be dependent on the diagnostic criteria of GDM and the postpartum follow-up period. Ethnicity also influences the risk of persistent pAGT[30]. Compared with Caucasian and Hispanic women, Asian women have a relatively low BMI, but show an increased propensity to develop prediabetes or diabetes. Therefore, Asian women might have impaired β-cell compensation as compared with women of other ethnicities when a similar extent of insulin resistance exists[16,17]. In the present study, metabolic parameters (1 h-PG, 2 h-PG, IGI and ISSI-2) of antenatal OGTT were associated with pAGT. These parameters are thought to be related to β-cell function. Therefore, the present results suggested that women with lower levels of antepartum β-cell function were at a higher risk of postpartum glucose intolerance.

The present study showed that the antepartum 2-h PG exhibited the largest AUC. In clinical practice, antepartum predictors for pAGT are useful because of a potential increase in GDM prevalence according to the IADPSG criteria. Until now, only a few reports have been published with regard to predictors of pAGT in women with GDM, as defined by the IADPSG criteria. Capula et al.[7] reported that 2-h PG in the antepartum OGTT was correlated with the development of prediabetes and type 2 diabetes mellitus within 1 year after delivery in cases of GDM, as defined by the IADPSG criteria. Benhalima et al.[13] showed that ethnicity and glycated hemoglobin at the time of OGTT during pregnancy were significant predictors of postpartum glucose intolerance in women with IADPSG criteria-based GDM. However, to the best of our knowledge, no reports on predictors of pAGT in Japanese GDM based on the IADPSG criteria are available. Therefore, further investigations are warranted to identify antenatal predictors of pAGT in Japanese women with GDM, as defined by the IADPSG criteria (i.e., WHO 2013 criteria).

The present study demonstrated that four SNPs showed a nominally significant association with the development of
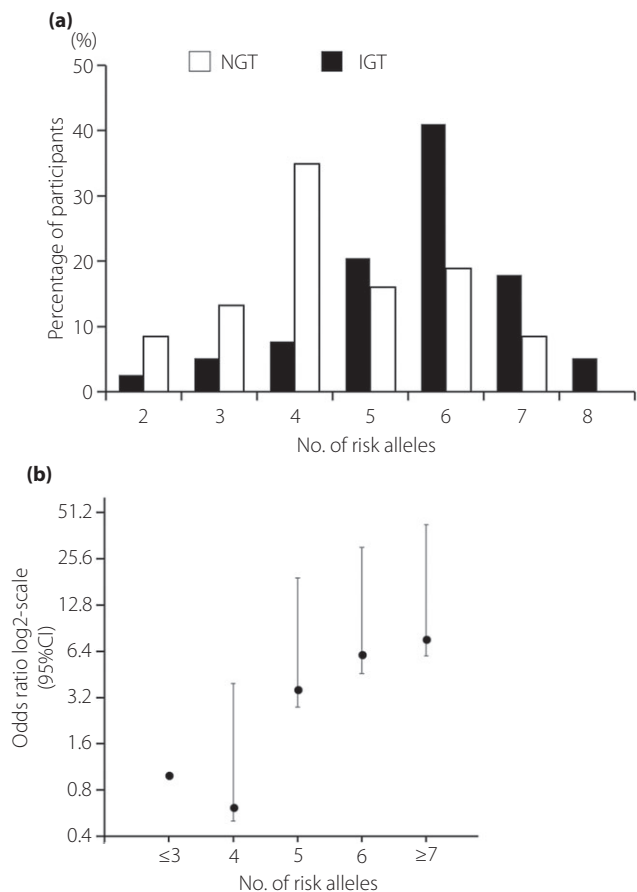
Figure 2 | Risk alleles of four genetic variants and the development of postpartum abnormal glucose tolerance (pAGT). (a) Distribution of risk alleles of four genetic variants in postpartum abnormal glucose tolerance and normal glucose tolerance (NGT). Black bars: abnormal glucose tolerance (n = 59); white bars: NGT (n = 154). (b) Odds ratio for the risk of postpartum abnormal glucose tolerance according to the number of risk alleles carried. 95% CI, 95% confidence interval; IGT, impaired glucose tolerance.

pAGT in women with recently developed IADPSG-defined GDM: rs266729 (*ADIPOQ*), rs6017317 (*HNF4A*), rs5215 (*KCNJ11*) and rs7177055 (*HMG20A*). Notably, women with an increasing number of risk alleles had a significantly higher risk of pAGT. Several authors have shown that *CDKN2A/2B*, *HHEX, CDKAL1, TCF7L2* and *FTO* carry genetic risk variants associated with pAGT in women with GDM, as defined by the Carpenter–Coustan or WHO 1999 criteria[9,14,15]. Our observation suggests that multiple genetic factors might contribute to the development of pAGT in Japanese women with GDM based on the IADPSG criteria.

Of the four genetic variants (four genes) identified in the present study, several studies showed that a single polymorphism, rs266729 (*ADIPOQ*), was associated with decreased levels of serum adiponectin in GDM, as well as type 2 diabetes mellitus[31]. Our previous investigation showed that rs266729

(*ADIPOQ*) was associated with Japanese GDM[28]. Furthermore, hepatocyte nuclear factor 4 alpha (*HNF4A*) is known as the gene responsible for maturity onset diabetes in the young population[32]. Although the mechanisms underlying the development of impaired insulin secretion remain unknown, an early hypersecretion of insulin *in utero* and in the neonatal periods, and pancreatic β-cell exhaustion later in life are suspected contributing factors, along with possible variations in gene expression over time[33]. *KCNJ11*, encoding a member of the potassium channel gene family, contributes to insulin secretion[34]. Previous reports showed that rs5215 is associated with the development of type 2 diabetes mellitus[35]. High mobility group protein 20A (*HMG20A*) was related to the development of type 2 diabetes mellitus in European individuals[36], and it might contribute to insulin secretion[37]. It is important that three variants (rs6017317 [*HNF4A*], rs5215 [*KCNJ11*] and rs7177055 [*HMG20A*]) identified in the present study were found to be insulin-secretion candidate genes. The genetic features found in this study are consistent with the fact that glucose intolerance in East Asian individuals, including Japanese individuals, is characterized as impaired insulin secretion (i.e., β-cell dysfunction)[38]. Based on these findings, impaired insulin secretion might play an important role in the pathophysiology of pAGT in Japanese women with GDM.

There were several limitations to the present retrospective study. First, 156 women (73%) underwent postpartum diabetic screening between 13-weeks and 1-year postpartum. Therefore, the clinical and genetic characteristics found in the present study were derived from women showing glucose intolerance within 1-year postpartum after delivery. Second, postpartum diabetes screening is not mandatory for all GDM women in our institutions. As the rate of postpartum diabetes screening was 48% in our experience, the study cohort in this investigation might have selection bias. However, antepartum metabolic features were comparable between GDM women with and without postpartum diabetes screening during the study period (data not shown). Thus, we speculate that women receiving postpartum follow up appeared similar to those without the test with regard to the degree of glucose intolerance during pregnancy. Third, we focused on only SNPs with MAF >30%, as the present study cohort constituted a relatively small panel. For example, the *FTO* gene, a risk variant related to type 2 diabetes mellitus in Japanese men as well as European people, was not included in this analysis, because the MAF was ≤30% in Japanese people. Therefore, we might have missed SNPs with MAF ≤30% that are associated with pAGT in Japanese women with recently developed GDM. It is also important to replicate and evaluate the current findings in another independent case–control set. Finally, we did not examine the genetic risk score, because the genetic variants detected showed a nominally significant association with pAGT in the present study. As reported by Kwak *et al.*[9], the use of a genetic risk score might improve prediction over clinical risk factors. Further research using a larger cohort is

required to determine genetic characteristics associated with pAGT in Japanese women with GDM.

In summary, we investigated the clinical and genetic features associated with pAGT in Japanese women with recently developed GDM, as defined by the IADPSG criteria (i.e., WHO 2013 criteria) for the first time. The clinical risk factors included 1-h PG, 2-h PG, IGI and ISSI-2 derived from the antepartum OGTT. Among genetic characteristics, four SNPs in four genes (*ADIPOQ*, *HNF4A*, *KCNJ11* and *HMG20A*) showed a nominally significant association with the development of pAGT. In particular, three variants were related to insulin secretion. Our results suggested that antepartum clinical and genetic characteristics related to impaired insulin secretion appeared to function as risk factors of pAGT in Japanese women with recently developed GDM. Further investigations are required to determine the predictors of pAGT after pregnancies with GDM.

DISCLOSURE
The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Primers designed by Assay Design Suite in the present study.

Table S2 | Linear regression analysis between four single-nucleotide polymorphisms and postpartum metabolic features.