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ORIGINAL ARTICLE

An epigenomic signature of postprandial hyperglycemia in peripheral blood leukocytes

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Postprandial hyperglycemia is known to be one of the earliest signs of abnormal glucose homeostasis associated with type 2 diabetes. This study aimed to assess clinical significance of a 1-h postprandial glucose level for the development of diabetes, and identify epigenetic biomarkers of postprandial hyperglycemia. We analyzed clinical data from the oral glucose tolerance tests for healthy subjects ($n = 4502$). The ratio (Glu60/Glu0) of 1-h glucose levels to fasting glucose levels was significantly associated with an insulin sensitive index (QUICKI, quantitative insulin sensitivity check index) ($\beta = 0.055$, $P = 1.25E - 04$) as well as a risk of future pre-diabetic and diabetic conversion. Next, DNA methylation profile analyses of 24 matched pairs of the high and low Glu60/Glu0 ratio subjects showed that specific DNA methylation levels in the promoter region of an olfactory receptor gene (olfactory receptor gene family10 member A4, OR10A4) were associated with the Glu60/Glu0 ratios ($\beta = 0.337$, $P = 0.03$). Moreover, acute oral glucose challenges decreased the DNA methylation levels of OR10A4 but not the global DNA methylation in peripheral leukocytes of healthy subjects ($n = 7$), indicating that OR10A4 is a specific epigenomic target of postprandial hyperglycemia. This work suggests possible relevance of olfactory receptor genes to an earlier molecular biomarker of peripheral hyperglycemia and diabetic conversion.

INTRODUCTION

Glucose homeostasis is regulated by glucose sensing in many different tissues.[1–3] Pancreatic beta cells secrete insulin by glucose sensing for the maintenance of blood glucose levels. Some reports suggest that taste receptors are involved in glucose sensing to regulate glucose uptake in a subset of cells within the gastrointestinal tract.[4,5] Pro-opiomelanocortin neurons also respond to an abnormal glucose level to drive glucose excitation by ATP-mediated closure of ATP-sensitive potassium channels.[6] As in pancreatic beta cells and the liver, immune cells have been implicated in the pathogenesis of diabetes and metabolic syndrome.[7–10] However, little is known about the molecular basis of leukocyte glucose sensing at the epigenomic level.

Oral glucose tolerance test (OGTT) is a convenient physiological test to assess beta cell function and insulin sensitivity in a single individual.[11–13] The OGTT is more powerful than the fasting plasma glucose (FPG) for diagnosing pre-diabetes as well as metabolic syndromes. Therefore, many population-based cohort studies include the OGTT as one of the key biochemical measurements to obtain donors' health status. Indeed, many diabetes studies have exploited metabolic changes in the levels of glucose and other metabolites at each time point during the OGTT. The most OGTT studies have used largely single-point glucose levels at either 0 h (Glu0) or 2 h (Glu120) after the acute oral glucose challenge. However, the clinical significance of the combined glucose levels of fasting (Glu0) and 1-h glucose levels (Glu60) has not been extensively studied.

Many cancers and other diseases have aberrant epigenetic changes in the genome.[14] DNA methylation changes often act as a molecular sensor for cellular adaptation in response to cellular or environmental stresses or stimuli.[15–17] We hypothesized that postprandial hyperglycemia or acute oral glucose challenge is related to epigenomic status in peripheral blood to sensitize leukocytes for glucose homeostasis. To find the epigenomic signatures of blood cells involved in glucose sensing or homeostasis on postprandial glucose challenge, we examined DNA methylation levels of peripheral blood DNA samples from healthy donors who had an oral intake of 75 g glucose. DNA methylation chip experiment and subsequent pyrosequencing for validation led to identify an olfactory receptor family gene (olfactory receptor gene family10 member A4, OR10A4) as a candidate target for glucose sensing in peripheral blood leukocytes. These results suggest that the present DNA methylation levels of OR10A4 may be an indicator of future hyperglycemia or diabetes conversion. This work would also facilitate the discovery of epigenomic biomarkers of diabetes as well as new target genes of diabetes and metabolic disorders.

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MATERIALS AND METHODS

Study subjects

This study included participants from the Korean Genome Epidemiology Study (Ansan-Ansung community-based cohort study), which is currently at the seventh 2-year follow-up phase since the baseline study was started in 2001. Its study design, sampling, concept and consent were described elsewhere.[18,19] From the cohort study participants, we selected subjects ($n = 4502$) who had the OGTT measurements at the baseline and the first two follow-up phases (Table 1). For the OGTT, subjects were given 75 g glucose dissolved in 300 ml water (Glucola; Allegiance Healthcare Corporation, McGaw Park, IL, USA) to drink within a period of 5 min. Blood samples were collected at 0, 1 and 2 h after glucose ingestion. Homeostasis Model Assessment (HOMA) was used to estimate insulin resistance (HOMA-IR: fasting serum insulin $(mU l^{-1}) \times FPG$ $(mmol l^{-1})/22.5$) and beta cell function (HOMA-beta: fasting serum insulin $(mU l^{-1}) \times 20/(FPG (mmol l^{-1}) - 3.5)$). Epidemiological and biochemical data including the OGTT measurements and DNA samples for this study were provided by the National Biobank of Korea and the Korean Genome and Epidemiology Study (KoGES) according to the approval of the sample and data access committee. The present study was approved by the institutional review board of Korea National Institute of Health.

DNA samples

For the DNA methylation analysis, 48 non-diabetic subjects were first selected to include 2 groups (24 subjects for the case group and 24 subjects for the control group) from the healthy subjects who remained normal blood glucose levels during the baseline and the first two follow-ups. These case and control groups represented 2.2–2.4 (high-ratio group, case) and 1.0–1.2 (low-ratio group, control) of the ratio of Glu60 to Glu0, respectively. Individuals of the case group were age-, sex- and body mass index (BMI ± 2)-matched with individuals of the control group. Each group consisted of equal numbers of male ($n = 12$) and female ($n = 12$). For the initial discovery set (Supplementary Table S1) using the genome-wide DNA methylation chip, two pairs of case ($n = 2$) and control ($n = 2$) subject groups were randomly selected from the case group ($n = 24$, high ratio of Glu60/Glu0) and control group ($n = 24$, low ratio of Glu60/Glu0). These two pairs were also age-, sex- and BMI(± 2)-matched.

DNA methylation chip experiment

Genomic DNA from fasting peripheral blood was used for the DNA methylation experiments for this study. High-quality genomic DNA (500 ng for each sample) was modified by sodium bisulfate using the EZ DNA methylation kit (Zymo Research, Orange, CA, USA) according to the manufacturer's instruction. The bisulfite-converted DNA was then used in the Infinium HumanMethylation27 BeadChip, as described in the manufacturer's instruction (Illumina, San Diego, CA, USA). The β-value reflects the methylation level of each CpG site. The β-value was calculated by subtracting background using negative controls on the array and taking the ratio of the methylated probe signal intensity to the total locus intensity of both methylated and unmethylated signals. A β-value of 0–1.0 represents significant percent methylation, from 0 to 100%, respectively, for each CpG site. Targets with a

Table 1 Baseline characteristics of study subjects with non-T2DM

Variables	Measurements (mean ± s.d.)
Total numbers (n)	4502
Male (*n*, %)	2171 (48.2)
Female (*n*, %)	2331 (51.8)
Age (years)	51.31 ± 8.54
Fasting glucose (mg dl^{-1})	81.27 ± 7.01
1 h glucose (mg dl^{-1})	135.92 ± 32.93
2 h glucose (mg dl^{-1})	104.00 ± 20.39
Fasting insulin (µIU ml^{-1})	7.53 ± 4.95
1 h insulin (µIU ml^{-1})	34.02 ± 32.62
2 h insulin (µIU ml^{-1})	25.70 ± 22.71

Abbreviation: T2DM, type 2 diabetes mellitus.

detection *P*-value of > 0.05 were excluded in 27 578 targets. Statistical significance of the methylation data was determined using the paired *t*-test, in which the null hypothesis was that no difference exists between mean of groups in the methylation data. False discovery rate was controlled by adjusting *P*-value using Benjamini–Hochberg algorithm. IlluminaBeadstudio v3.1 software (Illumina, San Diego, CA, USA) was used for quantification, image analysis of methylation data. R scripts were used for all other analytical process.

Pyrosequencing

Pyrosequencing primers were designed to amplify CpG dinucleotide sites in the target regions of genes using PSQ Assay Design software (Biotage AB, Sweden). Primer sequences and PCR condition are given in Supplementary Table S2. Bisulfite-treated DNA was amplified in a 50-µl reaction with the primer set and AmpliTaq polymerase (Applied Biosystems, Forster City, CA, USA). The PCR was performed at 95 °C for 5 min for denaturation, followed by 40 cycles at 95 °C for 90 s, 40–53 °C for 60 s, 72 °C for 45 s, and then a final extension at 72 °C for 7 min. The biotinylated PCR product was then purified by using Streptavidin-Sepharose beads (GE Healthcare Life Sciences, Buckinghamshire, UK). Pyrosequencing was performed using the PSQ96 SNP Reagent kit and the PSQ96 MA instrument as instructed by the manufacturer (Biotage, Uppsala, Sweden). Raw data were analyzed with the allele quantitation algorithm of the software (Pyro Q-CpG^{TM}SW).

RESULTS

Clinical significance of a postprandial 1-h glucose level

We noted the postprandial 1-h glucose level (Glu60) to find an earliest molecular sign of abnormal glucose homeostasis in peripheral leukocytes. To assess clinical significance of postprandial 1-h glucose level, we first used relative ratios of Glu60 to Glu0 (Glu60/Glu0) and absolute differences between Glu60 and Glu0 (Glu60 − Glu0) from OGTT measurements for healthy subjects ($n = 4502$) who had normal glucose levels at the baseline study. In the linear regression analysis with age and sex adjustments, we found that the Glu60/Glu0 ratio was significantly associated with an insulin sensitivity index (QUICKI (quantitative insulin sensitivity check index), $\beta = 0.055$, $P = 1.25E - 04$), but not with other metabolic indices (for example, HOMA-IR, HOMA-beta and fasting glucose to insulin ratio (FGIR)) (Table 2). In contrast, the Glu60 − Glu0 was associated particularly with beta cell function (HOMA-beta, $\beta = -0.054$, $P = 1.45E - 0.4$). In addition, the Glu60/Glu0 and the Glu60 − Glu0 showed a similar pattern in associations with postprandial 1-h and 2-h glucose or insulin levels as well as other metabolic parameters including triglyceride, C-reactive protein, glycosylated hemoglobin (HbA1C), BMI. In contrast, the fasting glucose and total cholesterol levels were associated only with the Glu60 − Glu0 delta change, but not with the Glu60/Glu0 ratio. Moreover, further analyses of the follow-up data for the non-diabetic subjects showed that the higher Glu60/Glu0 ratio is related to the higher risk of the future pre-diabetes or diabetes conversion about 4 years later (Supplementary Figure S1). Taken together, these results suggest that the Glu60/Glu0 ratio may provide a metabolic index to assess early abnormalities of glucose homeostasis when the Glu60 is combined with the Glu0, supporting the clinical importance of postprandial 1-h glucose levels.

DNA methylation sites associated with the Glu60/Glu0 ratio

To discover epigenomic signatures associated with the Glu60/Glu0 ratio, we analyzed genome-wide DNA methylation profiles of blood DNA samples from the discovery set, which included two pairs of the case (high Glu60/Glu0 ratio) and control (low Glu60/Glu0 ratio) subjects matched with age, sex and BMI(± 2). According to the detection *P*-value (adjusted $P < 0.05$) in the local pooled error test, we found 28 CpG sites including hypermethylated ($n = 20$) and

Table 2 Associations of OGTT measurements with the Glu60/Glu0 ratio or the Glu60 − Glu0 difference

Variables	Ratio (Glu60/Glu0)		Delta (Glu60 − Glu0)	
	Beta (β)[a]	Significance (P)	Beta (β)[a]	Significance (P)
Fasting glucose (mg dl^{-1})	−0.029	4.10E−02	0.104	**1.12E−13**
1 h glucose (mg dl^{-1})	0.939	**<1.00E−99**	0.979	**<1.00E−99**
2 h glucose (mg dl^{-1})	0.297	**<1.00E−99**	0.321	**<1.00E−99**
Fasting insulin (µIU ml^{-1})	−0.009	5.25E−01	0.006	6.57E−01
1 h insulin (µIU ml^{-1})	0.227	**1.95E−58**	0.239	**2.06E−64**
2 h insulin (µIU ml^{-1})	0.192	**5.79E−43**	0.195	**5.97E−44**
HOMA-IR[b]	−0.009	5.16E−01	0.023	1.12E−01
HOMA-beta[c]	−0.007	6.29E−01	−0.054	**1.45E−04**
QUICKI[d]	0.055	**1.25E−04**	0.021	1.32E−01
FGIR[e]	0.030	3.40E−02	0.025	8.40E−02
AUC glucose[f] (mg dl^{-1}) (h)	0.881	**<1.00E−99**	0.932	**<1.00E−99**
AUC insulin[g] (µIU ml^{-1}) (h)	0.247	**1.80E−69**	0.258	**7.89E−76**
Total cholesterol (mg dl^{-1})	0.034	1.70E−02	0.057	**5.84E−05**
High-density lipoprotein (mg dl^{-1})	0.014	3.12E−01	0.020	1.51E−01
Triglyceride (mg dl^{-1})	0.061	**1.58E−05**	0.068	**1.53E−06**
C-reactive protein, CRP (mg l^{-1})	0.052	**2.59E−04**	0.046	**1.00E−03**
HbA1C (%)	0.166	**3.20E−33**	0.194	**7.51E−45**
Body mass index (kg m^{-2})	0.049	**1.00E−03**	0.071	**6.37E−07**

Abbreviations: FGIR, fasting glucose to insulin ratio; HbA1C, glycosylated hemoglobin; OGTT, oral glucose tolerance test.
[a]Beta regression coefficient (adjusted with age and sex).
[b]Homeostasis model assessment-insulin resistance = fasting insulin concentration (µIU ml^{-1}) × fasting glucose concentration (mmol l^{-1})/22.5).
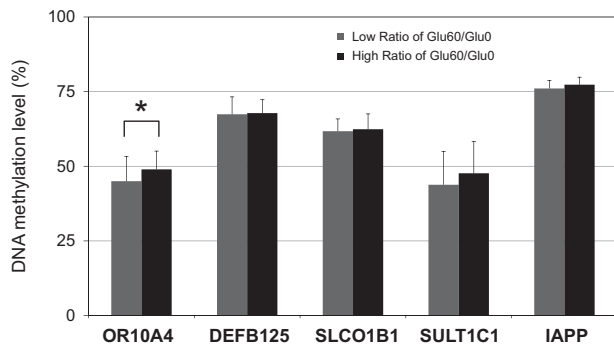[c]Beta cell function = 20 × fasting insulin (µIU ml^{-1})/(fasting glucose (mmol l^{-1}) − 3.5).
[d]Quantitative insulin sensitivity check index = 1/(log(fasting insulin)+log(fasting glucose)).
[e]Fasting glucose (mg dl^{-1}) to insulin (µIU ml^{-1}).
[f]Area under the curve of glucose levels during the OGTT.
[g]Area under the curve of insulin levels during the OGTT.
Bold letters indicate $P < 0.05$.

Figure 1 DNA methylation levels of selected CpG sites between the low- and high-ratio groups of Glu60/Glu0. Pyrosequencing was performed to target a particular promoter CpG site of each selected gene: *OR10A4* (cg03898365), *SULT1C1* (cg13968390), *IAPP* (cg15583072), *DEFB125* (cg08088390) and *SLCO1B1* (cg00995065). One promoter CpG site of *OR10A4* showed higher DNA methylation level in the high-ratio group of Glu60/Glu0 than in the low-ratio group of Glu60/Glu0, but not the other CpG sites. The data are represented as mean ± s.d. of three independent experiments (*n* = 24 in each group). *P = 0.068 (marginal significance), *t*-test. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

hypomethylated (*n* = 8) CpG sites in the high-ratio group of Glu60/Glu0 compared with the low-ratio group of Glu60/Glu0 (Supplementary Table S3, Supplementary Figure S2). These differential methylation sites included seven promoter CpG islands, one non-promoter CpG island, eleven promoter CpG sites and six non-promoter CpG sites.

Next, the same DNA methylation chip data were further used for inter-individual comparison, in which differentially methylated CpG

sites were identified from male or female pairs of low and high Glu60/Glu0 ratio subjects. According to the criteria of the delta change ($|\Delta\beta| > 0.06$) of DNA methylation levels of each probe, the male pairs represented hypomethylated (*n* = 1410) and hypermethylated (*n* = 1240) sites in a high Glu60/Glu0 subject in relative to a low Glu60/Glu0 subject whereas the female pairs represented hypomethylated (*n* = 1384) and hypermethylated (*n* = 1867) sites. Through these inter-group ($|\Delta\beta| > 0.13$) and inter-individual ($|\Delta\beta| > 0.06$) comparison of DNA methylation profiles, we finally selected five candidate differential DNA methylation sites in the promoter or non-promoter regions of *SULT1C1*, *OR10A4*, *DEFB125*, *SLCO1B1* and *IAPP* for further validation studies. All of these DNA methylation sites exhibited considerable changes ($|\Delta\beta| > 0.13$) in DNA methylation levels in the inter-individual comparison within the group (Supplementary Table S4).

Increased DNA methylation of OR10A4 gene in the high-ratio group of Glu60/Glu0

Pyrosequencing was performed to validate DNA methylation levels of candidate CpG sites in the validation sample set, which included case (*n* = 24) and control (*n* = 24) subjects matched with age, sex and BMI (±2). Among the five candidate CpG sites, the CpG site of *OR10A4* gene exhibited marginal significance (P = 0.068) of an increased DNA methylation level in the high-ratio group of Glu60/Glu0, compared with the low-ratio group of Glu60/Glu0 (Figure 1). In addition, we also observed a gender difference of the DNA methylation level in the CpG site of *OR10A4* gene. Regardless of the high or low-ratio group of Glu60/Glu0, DNA methylation levels of the CpG site of *OR10A4* gene were significantly higher (P = 0.019) in the male group, compared with the female group (Supplementary Figure S3).

Associations between OR10A4 DNA methylation levels and clinical variables

We conducted linear regression analysis to find some diabetes-related clinical variables associated with the DNA methylation levels of *OR10A4* gene. Among OGTT measurements from the validation set ($n = 48$), variables of Glu60 ($P = 0.056$), Glu60/Glu0 ($P = 0.030$) and Glu60/Glu120 ($P = 0.013$) ratios were associated with DNA methylation levels of *OR10A4* gene, but not those of Glu120/Glu0 (Table 3). In contrast, glucose homeostasis-related indices including HOMA-IR, HOMA-beta and QUICKI were not significantly associated with DNA methylation of *OR10A4* gene. However, the global DNA methylation showed no significant association with Glu60-related variables (data not shown). This result suggests that specific DNA methylation of *OR10A4* gene is associated with postprandial glucose levels in blood leukocytes.

Acute oral glucose challenge decreased the DNA methylation level of Or10A4 in leukocytes

For 7 subjects with normal glucose tolerance, the DNA methylation level of *OR10A4* was slightly decreased in peripheral blood leukocytes during time points (0, 1 and 2 h) of the OGTT (Figure 2). In particular, the acute oral glucose challenge decreased at the 1-h time point ($P = 0.08$), compared with the fasting time point, but significantly decreased DNA methylation level of *OR10A4* gene at the 2-h time point of OGTT ($P = 0.017$). In contrast, global DNA methylation in Alu, LINE-1 and SAT-α sequences showed no significant changes on the oral glucose challenge (data not shown). This result suggests that the CpG site of *OR10A4* gene is an epigenomic target of glucose sensing mechanism in peripheral blood leukocytes.

DISCUSSION

In an effort of epigenomic biomarker discovery for diabetes conversion, we focused on postprandial glucose levels in OGTT measurements. It is known that the 1-h postprandial glucose level (Glu60) provides useful metabolic information about glucose homeostasis.[20–22] Some reports showed that the Glu60 was associated with cardiovascular events, including carotid intima-media thickness[23] and left

ventricular hypertrophy.[24] To extend the understanding of the clinical significance of the 1-h glucose levels, we scrutinized the relative ratio of Glu60 to Glu0 (Glu60/Glu0) and the absolute difference between Glu60 and Glu0 (Glu60 − Glu0 delta change) rather than the Glu60 alone. Single glucose levels at individual time points of OGTT may be used as a possible index reflecting temporal human body's glycemic conditions on acute glucose challenges. However, such a single-point glucose level may not be sufficient to provide metabolic information about the systemic glycemia of the human body. Therefore, combined multi-point glucose levels of OGTT can provide more appropriate indices to assess glucose homeostatic potential of the human body.

In this study, we found associations of 1-h glucose levels alone, or combined fasting and 2-h glucose levels with glucose homeostasis-related indices (for example, insulin sensitivity or beta cell function). In particular, the Glu60/Glu0 ratio was associated with an insulin sensitivity index (QUICKI) but not with other metabolic indices. In contrast, the Glu60 minus Glu0 (Glu60 − Glu0) was associated with beta cell function (HOMA-beta) but not with QUICKI. Therefore, the present study implied that the relative ratio and the absolute difference of the Glu60 and Glu0 may reflect different physiological conditions of human body.

We reasoned that the relative ratio rather than the absolute difference of Glu60 and Glu0 is more likely attributed to the systemic glucose homeostasis of whole human body, which may depend on genetic make-ups or epigenetic regulation of individuals. Thus, we chose the relative ratios of two-point glucose levels (for example, 0-h and 1-h glucose levels) for further studies. To address the question what is the molecular basis of the relative Glu60/Glu0 ratio, we analyzed genome-wide DNA methylation profiles of the low- and high-ratio groups of Glu60/Glu0 to test the association between the Glu60/Glu0 and DNA methylation levels. As a result, the high ratio of Glu60/Glu0 was found to be associated with higher DNA methylation levels in *OR10A4*. In addition, the DNA methylation of *OR10A4* was decreased in peripheral leukocytes in response to the oral glucose challenge. This result suggests that an epigenetic change of *OR10A4* gene may be involved in glucose sensing in peripheral blood leukocytes. Thus, this is a possible link between an olfaction and systemic glucose homeostasis.

Table 3 Association of OR10A4 DNA methylation with Glu60-related variables

Variables	Beta (β)[a]	P-value[b]
Glu0	−0.158	0.323
Glu60	**0.298**	**0.056**
Glu120	−0.167	0.255
Ratio Glu60/Glu0	**0.337**	**0.030**
Ratio Glu120/Glu0	−0.110	0.468
Ratio Glu60/Glu120	**0.360**	**0.013**
Ins0	−0.175	0.264
Ins60	0.236	0.142
Ins120	−0.038	0.819
Hb1Ac	0.064	0.698
HOMA-IR	−0.193	0.218
HOMA-beta	−0.050	0.759
QUICKI	0.127	0.413
AUC glucose	0.233	0.139
AUC insulin	0.200	0.217

Abbreviations: AUC, area under the curve; HbA1C, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index.
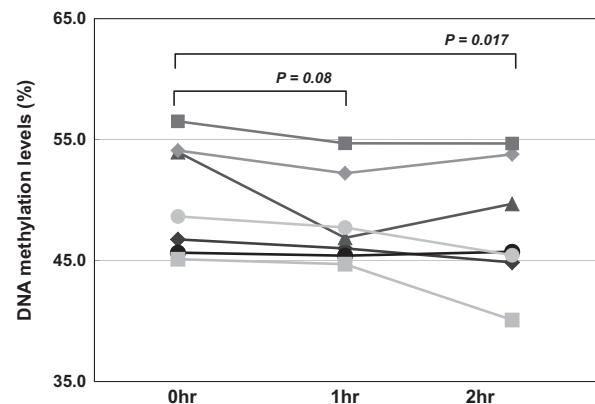[a]Beta regression coefficient.
[b]Age-, sex- and area-adjusted *P*-value.
Bold letters indicate $P < 0.05$.

Figure 2 The oral glucose tolerance test (OGTT) effect on DNA methylation levels of *OR10A4* gene. An oral glucose challenge decreased the DNA methylation level of the CpG site (cg03898365) of *OR10A4* gene in peripheral blood cells. The DNA methylation levels were quantified by pyrosequencing peripheral blood DNA samples from non-diabetic subjects ($n = 7$) with normal glucose tolerance in the OGTT time course (0, 1 and 2 h). *P*-values indicate the significance of paired *t*-test. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

The *OR10A4* gene is one of the largest gene family in the genome.[25] The olfactory receptor proteins, members of a G protein-coupled receptor family, are responsible for the recognition and G protein-mediated transduction of odorant signals. Recent studies showed that environmental and external cues act through sensory pathways to influence hormone secretion and glucose homeostasis.[26–28] For example, the taste receptors such as TAS1R and TAS2R detect sweet and bitter tasting stimuli to secrete signal transduction molecules immediately.[4] These taste receptors are known be an important modulator of insulin biosynthesis and secretion. On the other hand, the olfactory signaling occurs outside the olfactory epithelium, such as sperm[29] and kidney.[30] It is known that olfactory function is reduced in aged humans and diabetes mellitus patients.[31] For example, an olfactory dysfunction was significantly associated with higher HbA1c and FPG. Therefore, it is conceivable that an olfactory machinery may be expressed in leukocytes to have a role in blood glucose sensing or homeostasis, which may be linked to inflammatory pathways.

Aberrations in the methylation pattern have been shown to contribute to cancer and various diseases. In the present study, we showed that peripheral blood cells responded to change DNA methylation levels in the promoter region of *OR10A4* gene in OGTT. In fact, an acute oral glucose challenge decreased in DNA methylation levels in the promoter region of *OR10A4* gene, but not in the global DNA methylation level, suggesting a possible role of specific DNA methylation of *OR10A4* gene for glucose sensing in peripheral blood leukocytes.

On the other hand, we observed a higher DNA methylation level of *OR10A4* in men than in women. In previous studies, the middle-aged men were at significantly higher risk of diabetes than women in several different populations.[32–34] It was also reported that men were diagnosed with type 2 diabetes at a lower BMI than women across the age range.[35] The development of type 2 diabetes is caused by multiple factors, including lifestyle, age and genetics. In our work, the gender difference of *OR10A4* DNA methylation patterns implies that a higher DNA methylation of *OR10A4* could attribute to a higher risk of diabetes progression or abnormal glucose homeostasis in men than in women. However, it remains to be further investigated the gender effect of *OR10A4* DNA methylation on glucose sensing and diabetes progression.

The present study has some limitations. A first one is that this study used DNA samples from human peripheral blood cells to discover the epigenomic biomarker of postprandial hyperglycemia. The peripheral blood leukocyte may not be the ideal target tissue to study diabetogenesis, but it would be the best tissue for the discovery of diagnostic and therapeutic targets of diabetes. First, for example, blood cells are not only easier to obtain test samples than diabetes target tissues (for example, pancreas, liver and muscle), but also have shown to reflect transcriptomic and epigenomic profiles of the target tissues.[36–39] Second, there are a number of reports that metabolic diseases such as diabetes and obesity are associated with leukocyte-mediated inflammatory processes.[40–42] Third, genome-wide association studies that used DNA from blood cells have shown the clinical utility of epigenomic biomarkers as diagnostic and therapeutic targets of diabetes.[43–45] A second limitation is that there are insufficient numbers of participants for the present epigenomic study. This study used age-, sex- and BMI-matched case–control samples for the discovery set, and subsequently random peripheral blood cells to validate the epigenomic change of *OR10A4* at three points (0, 1 and 2 h) of OGTT. However, further studies remain to be investigated for the replication study using large-scale population-based cohorts as well

as the *in vitro* study to demonstrate biological roles of DNA methylation of *OR10A4* in peripheral leukocytes.

In conclusion, a higher ratio of Glu60/Glu0 was associated not only with insulin sensitivity index (for example, QUICKI) in non-diabetic subjects, but also with a higher risk of the future diabetes progression from normal glucose tolerance, supporting the clinical importance of postprandial 1-h glucose levels giving more metabolic information. The higher Glu60/Glu0 ratio was also associated with a higher DNA methylation level of *OR10A4*, suggesting that *OR10A4* is an epigenomic target of postprandial hyperglycemia in leukocytes. Accordingly, the epigenomic change of *OR10A4* gene may be involved in the glucose sensing and homeostasis. It will be interesting to assess a predictive power of DNA methylation levels of *OR10A4* for the risk of diabetes progression in a large sample size. Our work would provide a clue to find new target genes to connect olfactory receptors to diabetogenesis and metabolic disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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