SUPPLEMEMTARY DATA

Methylation Status of Vault RNA 2-1 Promoter Is a Predictor of Glycemic Response to Glucagon-like Peptide-1 Analogue Therapy in Type 2 Diabetes Mellitus

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Supplemental Table S1 Demographic variables of 10 participants (training group) applied for DNA methylation chip at baselines

Variables	Responsive *n*=7	Non-responsive *n*=3	*P*
Age (years)	56.6 ±10.0	46.0±21.6	0.302
Sex (female %)	28.6	33.3	0.301
BMI	27.8±5.5	32.1±3.8	0.760
DM duration (years)	11.8 ± 7.8	10.0 ± 2.6	0.345
HbA1c at baselines (%)	11.1 ± 1.9	9.5 ± 0.7	0.358
Comorbidity (%)			
Hypertension	71.4	66.7	0.880
Hyperlipidemia	71.4	66.7	0.880
Chronic kidney disease	57.1	33.3	0.490
Cardiovascular disease	14.3	33.3	0.490
Concurrent therapy (%)			
Statins	42.9	0	0.475
ACEI/ARB	14.3	66.7	0.098
CCB	57.1	33.3	0.490
Beta-blocker	28.6	33.3	0.880
Insulin	100	100	-
Sulfonylurea	0	0	-

Thiazolidinedione	0	0	
Metformin	0	0	
Acarbose	0	0	
Glinide	0	0	-

ACEI/ARB : Angiotensin- converting enzyme inhibitor/ Angiotensin receptor blocker

CCB: Calcium channel blocker

Supplemental Table S2 Demographic variables of participants (validation group) enrolled in this study

Variables	Responsive *n*=93	Non-responsive *n*=35	*P*
Age	54.6 ±13.9	50.7 ±13.7	0.189
Sex (female %)	47.3	58.3	0.326
BMI	27.9 ±5.3	28.6 ±5.2	0.563
DM duration (years)	12.3 ± 1.4	11.6 ± 3.7	0.684
HbA1c before GLP-1 (%)	10.4 ± 0.3	10.3 ± 0.5	0.875
HbA1c after GLP-1 (%)	8.6 ± 0.5	11.1 ± 0.2	< 0.0001***
Comorbidity (%)			
Hypertension	62.5	42.9	0.447
Hyperlipidemia	75.0	42.9	0.205
Chronic kidney disease	62.5	44.3	0.737
Cardiovascular disease	9.4	5.3	0.597
Concurrent therapy (%)			

Statins	62.5	28.6	0.189
ACEI/ARB	50.0	28.6	0.398
CCB	62.5	28.6	0.189
Beta-blocker	12.5	14.3	0.919
Insulin	67.9	77.8	0.357
Sulfonylurea	43.8	44.4	0.973
Thiazolidinedione	0	0	-
Metformin	87.5	100	0.245
Acarbose	12.5	10.0	0.846
Glinide	12.5	20.0	0.606

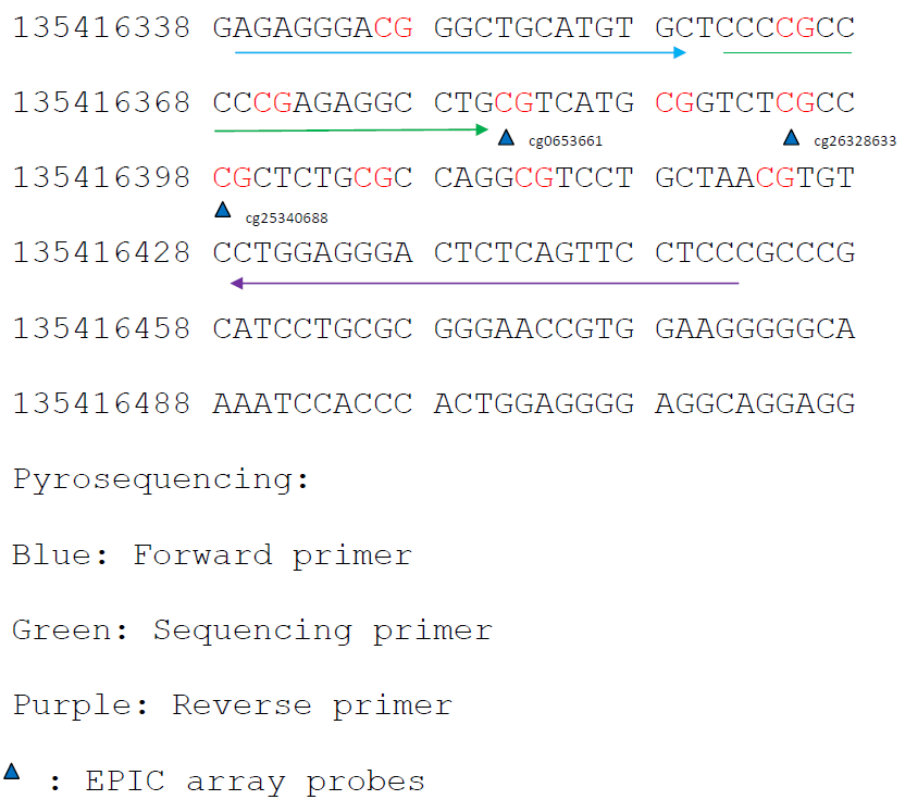
ACEI/ARB : Angiotensin- converting enzyme inhibitor/ Angiotensin receptor blocker

CCB: Calcium channel blocker

***$P<0.001$

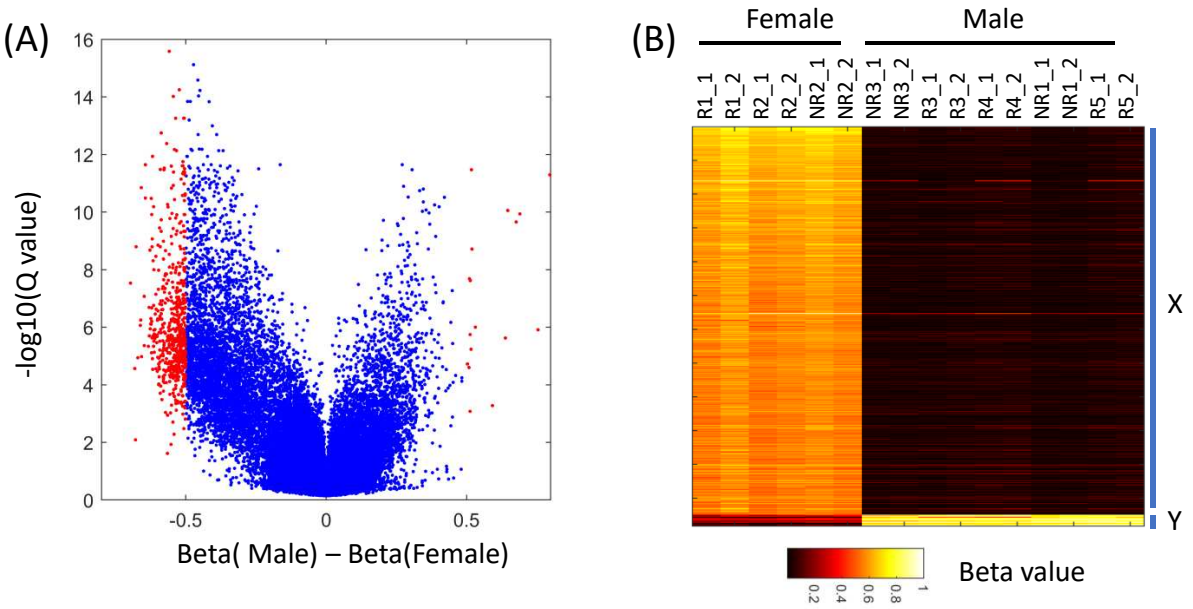
Supplement Table S3 Primers used for bisulfite sequencing primers (BSP), pyrosequencing reaction and centromeric CTCF (rs2346018) polymorphism

Gene Name	**Sense 5'-3'**	**Antisense 5'-3'**	**Size(bp)**	**Position (GRCh37/hg19 Assembly)**
VTRNA2-1 BSP	TTGAAATTTTTAAATTATAG AAGAGTGA	ATAAATAAATTTTACCC CCTTCCAC	197	chr5:135,416,303-13 5,416,499
Pyrosequencing-VTRNA2-1	AGAGGGAAGGGTTGTATGTGT	AAAAAAACTAAAAATCC CTCCAA (Biotin)	113	chr5:135,416,339-1 35,416,451
Pyrosequencing-VTRNA2-1 Sequencing primer	TTTAGTTTTAGAGAGGTTTG			Chr5:135,416,358-135,416,380
Centromeric CTCF site (rs2346018)	ATGGTGGTACCAGAGCAAGG	GAGAAAATGCCTGCGTC TCT	178	chr5:136,079,522-1 36,079,699

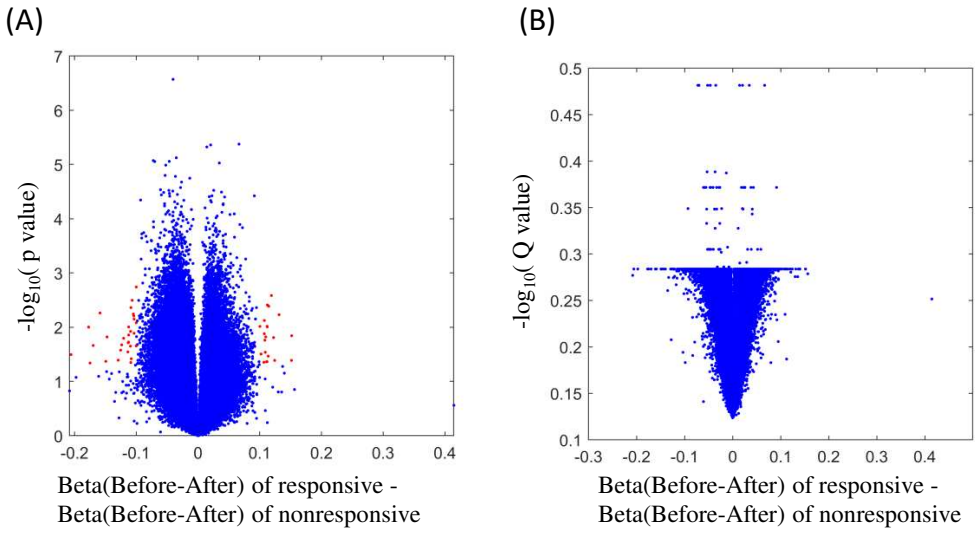


Supplemental Table S4 Differentially methylated regions (DMRs) in human genome and association with glycemic response of GLP-1 treatment in training group

Chromosome	Start	End	Width (bp)	*P*	Genes
chr5	135415693	135416613	920	< 0.0000000001	VTRNA2-1
chr2	113992694	113993313	619	0.00002	PAX8
chr8	143751533	143751796	263	0.00002	PSCA
chr16	70472993	70473294	301	0.00002	ST3GAL2
chr1	205819179	205819609	430	0.00004	PM20D1
chr6	29648161	29649092	931	0.00008	ZFP57
chr20	2187237	2187844	607	0.0001	LINC00654
chr6	31275267	31276669	1402	0.0006	HLA-B
chr13	23309689	23310675	986	0.0006	-
chr1	202310824	202311492	668	0.0007	UBE2T

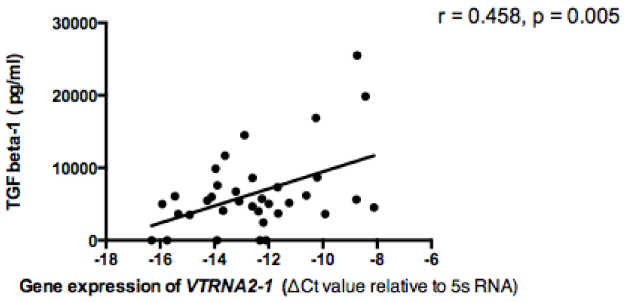


Supplement Figure S1 A gender difference was identified in Infinium® MethylationEPIC BeadChip analysis in the training group of this study through (A) volcano plot and (B) heatmap plot. (A) Volcano plot, X-axis: different of beta value between male and female, Y-axis: the -$\log_{10}$ Q value. Q value was the false discovery rate adjusted p value of the *t* test by comparing the means of beta value between male and female. The probe with Q value < 0.05 and difference of beta value between male and female > 0.5 were labeled with red spot. (B) Heatmap plot, differential expression probes labeled with red spots were plotted. The color map of the beta value was indicated. The probes located at X any Y chromosome were illustrated. Each number indicates the enrolled patients. The number-1 indicated genomic DNA extracted before GLP-1 analogue therapy; whereas number-2 indicated the genomic DNA extracted after GLP-1 analogue treatment.



Supplement Figure S2 The volcano plots of the differentially methylation comparing responsive and non-responsive groups before and after GLP-1 analogue treatment. The X- axis: the difference between responsive and non-responsive of the beta value before and after GLP-1 analogue treatment. The Y-axis: the $-\log_{10}$ p value at (A), and the $-\log_{10}$ Q value at (B). The p value was the *t* test comparing the means of beta value between responsive and non-responsive groups. The Q value was the false discovery rate adjusted p value. There was no differentially methylated region detected by the multiple test adjusted Q value.

(A)



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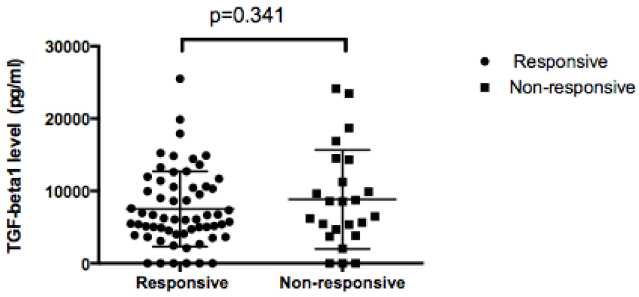

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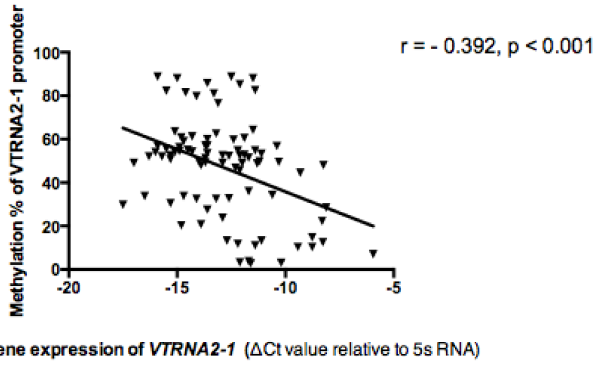
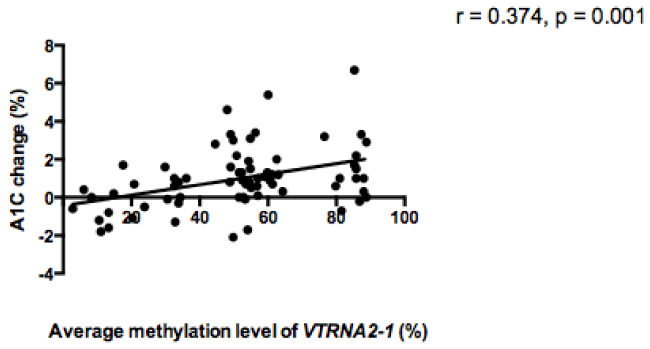


Supplemental Figure S3 Serum level of TGF-β1 as a predictor of a glycemic response to GLP-1 analogue treatment and the correlation of *VTRNA2-1* RNA expression versus its promoter methylation status. (A) Correlation of the gene expression of *VTRNA2-1* and serum level of TGF-β1 levels. (B) The serum level of TGF-β1 as a predictor for a glycemic response to GLP-1 analogue treatment. (C) Correlation between the *VTRNA 2-1* mRNA expression and methylation status of *VTRNA 2-1* promoter region.

(A)



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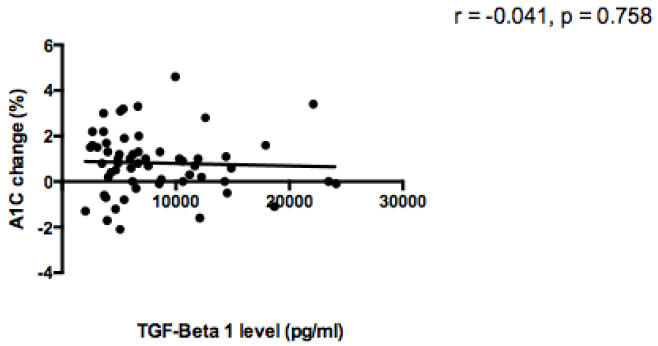

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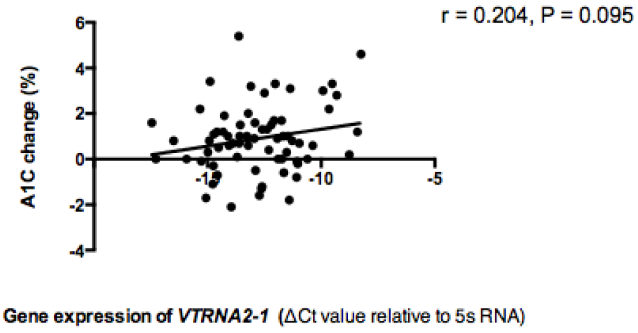

Supplement Figure S4 The association between change in A1C and (A) *VTRNA 2-1* methylation level, (B) TGF-beta1 levels, and (C) mRNA levels of *VTRNA 2-1*.