

Association Between Transcription Factor 7-Like-2 Polymorphisms and Type 2 Diabetes Mellitus in a Ghanaian Population

Supplementary Appendix 1

Text Summary

The supplementary material contains detailed information on the modified and simplified salting-out technique for DNA extraction (pp.2-3). Quality control and troubleshooting procedures for the genotyping of the SNPs have been shown on page 4-5. Figure S1 and S2 shows the results of DNA purity and concentrations. Figure S3 and S4 demonstrate the genotyping results of the two SNPs. Table S1 shows the recipe of the master mix for Quality Control and trouble shooting. Table S2-S5 shows the association between rs12255372 and rs7903146 with cardiometabolic risk factors in both cases and control participants. Supplementary appendix two shows details of logistic regression models used to examine associations.

All images are in Joint Photographic Experts Group (Jpeg) format.

Modified Salting-Out Non-enzymatic DNA extraction Method

Erythrocyte Lysis Buffer

Add 9 volume of 0.155M NH₄Cl (8.291g NH₄Cl into 1L dH₂O) to 1 volume of 0.17M Tris HCL (pH= 7.65)

Wash Buffer

Mix 5 ml of 1.0M NaCl and 10 ml of 0.5M EDTA (pH=8.0) into 985 mL dH₂O

TKM 2 (High salt buffer) Preparation (100 ml)

0.121g of Tris HCL (10mM, pH=7.6), 0.074g of KCl (10mM), 1.203g of MgCl₂ (10mM), 0.074g of EDTA (2mM), 0.467g of NaCl (0.4M) is dissolved in 100ml of dH₂O.

Saturated Sodium chloride (6M NaCl) solution

Dissolve 8.765g of NaCl in 25 ml of dH₂O

Steps

In a nuclease-free tube (Eppendorf Tube) add 3:1 ratio of Erythrocyte Lysis Buffer to whole blood sample (at 37 °C temperature). Incubate the mixture for 5 minutes. Centrifuged the mixture at 8000 rpm for 3 minutes. Discard the supernatant and keep the sediment. To ensure complete removal of haemoglobin stain, add 600 µl of wash buffer to the sediment and centrifuge the mixture at low speed (6000 rpm) for 5 minutes. Discard the supernatant. Resuspend the white cell pellet with 300 µl of TKM-2 buffer and add 40 µl of 10% SDS. Mix thoroughly by 30-secs vertexing and incubate at 37 °C for 5 minutes. At the end of the incubation, add 100 µl of 6M NaCl and vortex for 15 seconds to precipitate the proteins and leave DNA out in solution. Centrifuge the content at 8000 rpm for 5 minutes. Transfer the supernatant into a new Eppendorf tube containing 300 µl of absolute isopropanol (99.9%). Invert the tube slowly several times to precipitate DNA. Centrifuge at 8000 rpm for 10 minutes to pellet down the precipitated DNA. Discard the supernatant and add 70% isopropanol, mix slowly to remove any excess salts. Final centrifugation is conducted at 8000 rpm for 5 minutes to pellet down the DNA. Discard the supernatant. Place Eppendorf tube downward on the tissue paper to drain the ethanol completely. Air-dry the DNA and after thorough drying, add 50 µl of nuclease-free water buffer to dissolve the DNA.

Results visualisation of DNA products isolated with MSO method

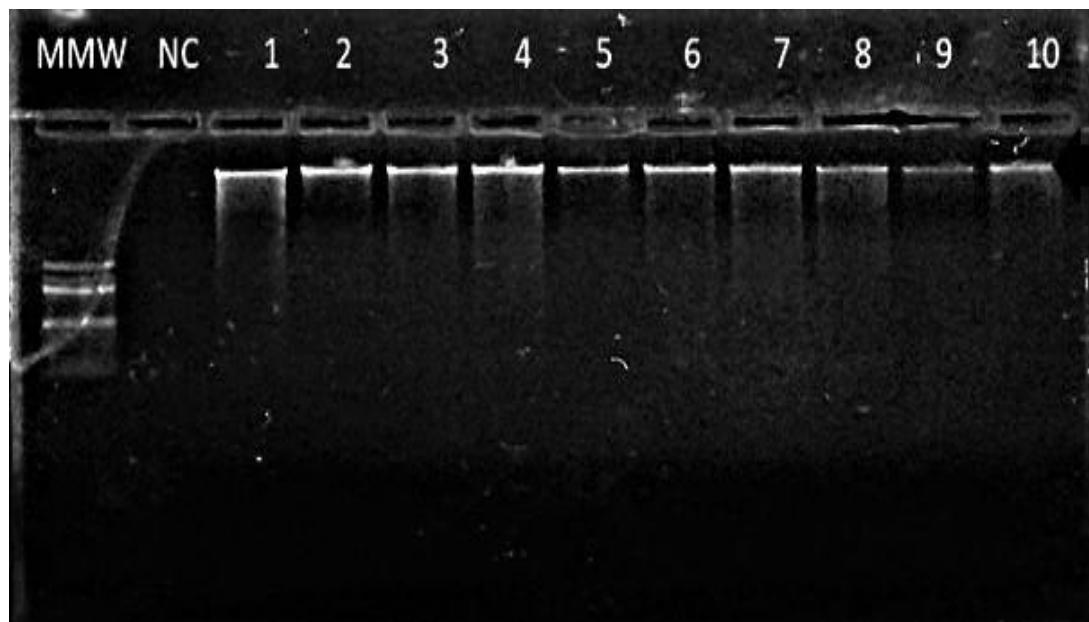


Figure S1: 1% agarose gel electrophoresis of genomic DNA extracted from MSO method

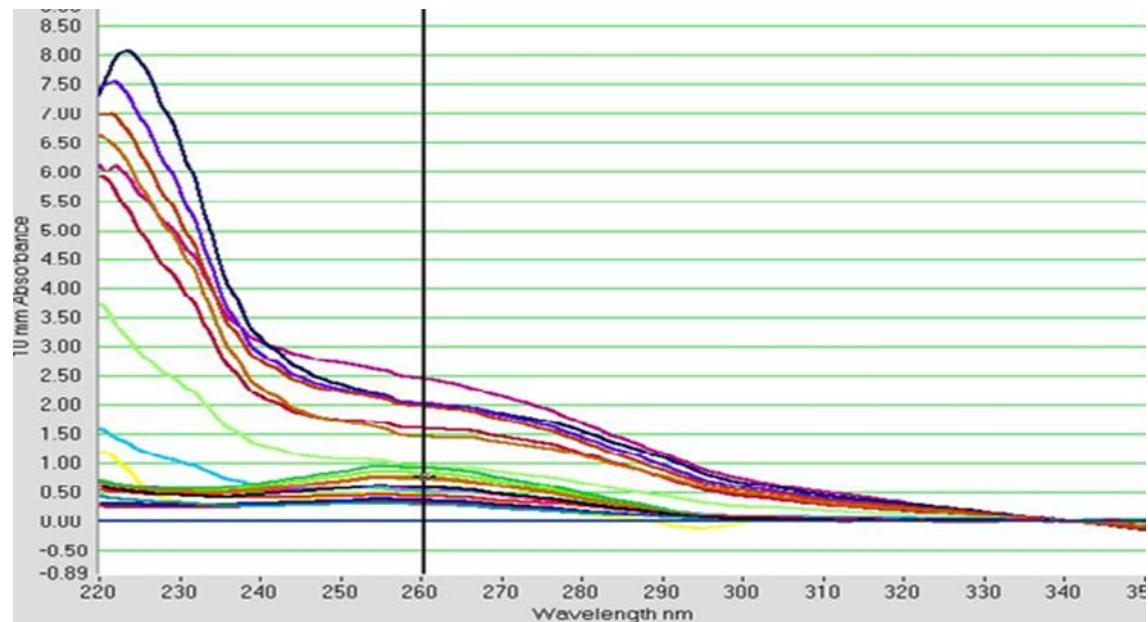


Figure S2: A typical Nanodrop curve of absorbance against the path of light travel (wavelength)

MTPA Quality control and Trouble Shooting

The optimization step of the MTPA solely depends on the primer concentration. Where appropriate, increase the Mg⁺⁺ concentration of the commercially prepared OneTaq® 2X Master Mix (New England Biolabs) by 0.2 μM during the optimization step.

Amplification of common product

Amplification of common primers was performed in a total volume of 25 μL containing ≥50 ng of DNA template, 0.4 μM each of forward and reverse outer primers and 1X of OneTaq® 2X Master Mix (New England Biolabs).

MTPA reaction

Allele-specific Amplification

Forward outer vs reverse inner primers; and Forward inner vs reverse outer primer combinations were used to optimise the allele-specific amplification conditions. The reaction was completed with 0.4 μM each of forward-outer vs reverse-inner primers in 25 uL reaction volume using the 1X of OneTaq® 2X Master Mix (New England Biolabs).

Table S1: Recipe for the master mix for Quality Control

Component	Initial Conc.	Final conc.	Volume (μL) Per Sample
Amplification of common Primers			
FOP	10 μM	0.4 μM	1
ROP	10 μM	0.4 μM	1
MTPA reaction			
FIP	10 μM	0.2 μM	0.5
RIP	10 μM	0.2 μM	0.5
FOP	10 μM	0.2 μM	0.5
ROP	10 μM	0.2 μM	0.5
Amplification of mutant Allele			
FIP	10 μM	0.4 μM	1
ROP	10 μM	0.4 μM	1
Amplification of ancestral Allele			
RIP	10 μM	0.2 μM	1
FOP	10 μM	0.4 μM	1
Onetaq® 2x Master Mix	2X	1X	12.5
Nuclease Free water	-	-	
DNA sample	>50 ng/ μL	>50 ng/ μL	1

FIP- forward inner primer; RIP- reverse inner primer; FOP; forward outer primer; ROP: reverse outer primer

Results of TCF7L2- rs7903146 and rs12255372 genotyping



Figure S3: 2% agarose gel electrophoresis of TCF7L2- rs7903146



Figure S4: 2% agarose gel electrophoresis of TCF7L2- rs12255372

Table S2: Association of TCF7L2 SNPs with Anthropometric and biochemical markers

Variables	Wild type	Mutant type	P-value
rs7903146			
Controls (N=110)			
BMI (Kg/m ²)	26.14±5.40	26.07±3.45	0.924
Systolic BP (mmHg)	120.96±19.15	121.49±17.96	0.142
Diastolic BP (mmHg)	75.57±11.11	76.74±11.28	0.403
Triglycerides (mmol/l)	1.31±0.33	1.27±0.61	0.048
T. Chol (mmol/l)	4.52±0.98	4.76±1.13	0.465
HDL-C(mmol/l)	1.24±0.23	1.22±0.30	0.163
LDL-C (mmol/l)	3.19±0.50	3.42±0.85	0.001
FPG (mmol/l)	4.63±0.75	5.02±0.98	0.069
HbA1c (%)	4.81±0.52	4.85±0.66	0.981
Cases (N=106)			
BMI (Kg/m ²)	28.62±5.71	28.04±5.49	0.160
Systolic BP (mmHg)	138.19±21.46	131.78±19.05	0.015
Diastolic BP (mmHg)	84.65±12.93	83.36±11.39	0.450
Triglycerides (mmol/l)	1.53±0.46	1.74±0.68	0.284
T. Chol (mmol/l)	4.95±0.94	5.35±1.04	0.110
HDL-C(mmol/l)	1.06±0.22	0.98±0.24	0.283
LDL-C (mmol/l)	3.31±0.72	3.48±1.01	0.046
FPG (mmol/l)	7.38±4.52	9.16±4.64	0.414
HbA1c (%)	6.23±1.49	7.24±1.92	0.099
rs12255372			
Controls (N=110)			
BMI (Kg/m ²)	25.72±4.64	27.17±3.53	0.493
Systolic BP (mmHg)	121.56±17.90	120.41±21.10	0.145
Diastolic BP (mmHg)	76.00±11.29	76.83±10.98	0.402
Triglycerides (mmol/l)	1.20±0.36	1.54±0.72	0.003
T. Chol (mmol/l)	4.51±1.03	5.04±1.07	0.048
HDL-C(mmol/l)	1.24±0.26	1.21±0.31	0.122
LDL-C (mmol/l)	3.27±0.61	3.45±0.99	0.001
FPG (mmol/l)	4.67±0.71	5.34±1.19	0.005
HbA1c (%)	4.72±0.55	5.17±0.63	<0.001
Cases (N=106)			
BMI (Kg/m ²)	27.54±6.00	29.05±4.75	0.044
Systolic BP (mmHg)	128.57±17.09	139.82±21.41	0.003
Diastolic BP (mmHg)	81.61±10.69	86.49±12.60	0.077
Triglycerides (mmol/l)	1.63±0.57	1.77±0.71	0.476
T. Chol (mmol/l)	5.30±1.09	5.18±0.95	0.339
HDL-C(mmol/l)	1.02±0.26	0.96±0.19	0.459
LDL-C (mmol/l)	3.48±0.99	3.38±0.89	0.061
FPG (mmol/l)	8.47±4.31	9.06±5.12	0.935
HbA1c (%)	6.69±1.52	7.40±2.21	0.078

Mt- mutant type, Wt- wild type. Highlighted P-values are statistically significant.

Table S3: Logistic regression analysis of TCF7L2 SNPs associated with cardiometabolic risk factors

Variables	Wild type (C-allele)	Mutant type (T-allele)	aOR (95%CI)	P-value
rs7903146				
Controls (N=110)				
High BP	4 (8.2)	6 (9.8)	0.991 (0.25-3.96)	0.990
High TC	1 (2.0)	5 (8.2)	5.14 (0.57-46.67)	0.146
High TG	7 (14.3)	9 (14.8)	0.93 (0.31-2.77)	0.890
Low HDL-C	24 (49.0)	28 (45.9)	1.12 (0.46-2.73)	0.800
High LDL-C	11 (22.4)	25 (41.0)	2.98 (1.23-7.30)	0.017
Atherogenic dyslipidaemia	1 (2.0)	4 (6.6)	3.71 (0.39-35.43)	0.254
Overweight/Obesity	23 (46.9)	37 (60.7)	1.73 (0.78-3.76)	0.164
MetS	6 (12.2)	8 (13.1)	1.02 (0.32-3.28)	0.969
Cases (N=106)				
High BP	11 (42.3)	25 (31.3)	0.62 (0.25-1.55)	0.307
High TC	1 (3.8)	12 (15.0)	4.46 (0.55-36.17)	0.162
High TG	10 (38.5)	51 (63.7)	2.95 (1.16-7.50)	0.023
Low HDL-C	18 (69.2)	63 (78.8)	1.73 (0.62-4.83)	0.299
High LDL-C	13 (50.0)	51 (63.7)	1.95 (0.75-5.10)	0.174
Atherogenic dyslipidaemia	7 (26.9)	37 (46.3)	2.67 (0.95-7.52)	0.063
Overweight/Obesity	16 (61.5)	56 (70.0)	1.48 (0.58-3.79)	0.405
MetS	15 (57.7)	55 (68.8)	1.70 (0.66-4.39)	0.272
rs12255372				
Controls (N=110)				
High BP	6 (7.4)	4 (13.8)	1.48 (0.35-6.45)	0.597
High TC	2 (2.5)	4 (13.8)	14.0 (1.43-137.32)	0.023
High TG	9 (11.1)	7 (24.1)	1.96 (0.64-6.02)	0.239
Low HDL-C	39 (48.1)	13 (44.8)	1.41 (0.48-4.18)	0.533
High LDL-C	23 (28.4)	13 (44.8)	3.12 (1.14-8.56)	0.027
Atherogenic dyslipidaemia	4 (4.9)	1 (3.4)	0.65 (0.08-6.30)	0.710
Overweight/Obesity	42 (51.9)	18 (62.1)	1.55 (0.62-3.84)	0.348
MetS	7 (8.6)	7 (24.1)	2.91 (0.88-9.59)	0.080
Cases (N=106)				
High BP	15 (24.6)	21 (46.7)	3.01 (1.27-7.18)	0.013
High TC	8 (13.1)	5 (11.1)	0.91 (0.27-3.09)	0.883
High TG	31 (50.8)	30 (66.7)	1.89 (0.83-4.32)	0.131
Low HDL-C	45 (73.8)	36 (80.0)	1.46 (0.56-3.88)	0.439
High LDL-C	36 (41.0)	28 (62.2)	1.24 (0.53-2.94)	0.622
Atherogenic dyslipidaemia	21 (34.4)	23 (51.1)	2.17 (0.91-5.17)	0.082
Overweight/Obesity	36 (59.0)	36 (80.0)	3.33 (1.30-8.53)	0.012
MetS	37 (60.7)	33 (73.3)	2.04 (0.84-4.97)	0.117

Mt- mutant type, Wt- wild type. Highlighted P-values are statistically significant. aOR-adjusted for age and sex

Supplementary Appendix 2

Logistic regression analysis to test association between rs7903146 and T2DM

Block 0: Beginning Block

		Classification Table ^{a,b}		Predicted T2DM Status	Percentage Correct
Observed		0	1		
Step 0	T2DM Status	0	110	0	100.0
		1	106	0	.0
Overall Percentage					50.9

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-.037	.136	.074	1	.786

Variables not in the Equation

	Variables	Score	df	Sig.
Step 0	rs7903146	12.066	2	.002
	rs7903146(1)	2.832	1	.092
	rs7903146(2)	5.049	1	.025
Overall Statistics		12.066	2	.002

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	12.354	2	.002
	Block	12.354	2	.002
	Model	12.354	2	.002

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	287.012 ^a	.056	.074

a. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	.000	1	1.000

Contingency Table for Hosmer and Lemeshow Test

		T2DM Status = 0		T2DM Status = 1		Total
Observed	Expected	Observed	Expected	Observed	Expected	
Step 1	1	49	49.000	26	26.000	75

2	56	56.000	66	66.000	122
3	5	5.000	14	14.000	19

Classification Table^a

Observed	T2DM Status	Predicted		Percentage Correct
		0	1	
Step 1	T2DM Status	0	49	61
		1	26	80
Overall Percentage				59.7

a. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs7903146			11.467	2	.003
	rs7903146(1)	.798	.303	6.931	1	.008
	rs7903146(2)	1.663	.575	8.376	1	.004
	Constant	-.634	.243	6.822	1	.009

Variables in the Equation

Step 1 ^a	rs7903146	95% C.I. for EXP(B)	
		Lower	Upper
	rs7903146(1)	1.226	4.023
	rs7903146(2)	1.711	16.277
	Constant		

a. Variable(s) entered on step 1: rs7903146.

Block 2: Method = Enter

Omnibus Tests of Model Coefficients

	Chi-square	df	Sig.
Step 1	Step	1.468	1
	Block	1.468	1
	Model	13.821	.003

Model Summary

Step	-2 Log likelihood	Cox & Snell R	Nagelkerke R
		Square	Square
1	285.544 ^a	.062	.083

a. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	.255	3	.968

Contingency Table for Hosmer and Lemeshow Test

	T2DM Status = 0		T2DM Status = 1		Total
	Observed	Expected	Observed	Expected	
Step 1	1	35	34.007	15	50
	2	14	14.993	11	25
	3	36	36.043	37	73

4	20	19.957	29	29.043	49
5	5	5.000	14	14.000	19

Classification Table^a

Observed	T2DM Status	Predicted		Percentage Correct
		0	1	
Step 1	0	49	61	44.5
	1	26	80	75.5
Overall Percentage				59.7

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs7903146		10.856	2	.004	
	rs7903146(1)	.779	.304	1	.010	2.180
	rs7903146(2)	1.625	.577	1	.005	5.081
	Sex(1)	-.350	.289	1.464	1	.226
	Constant	-.404	.307	1.739	1	.187

Variables in the Equation

			95% C.I. for EXP(B)	
			Lower	Upper
Step 1 ^a	rs7903146			
	rs7903146(1)		1.201	3.959
	rs7903146(2)		1.640	15.738
	Sex(1)		.400	1.242
	Constant			

Block 3: Method = Forward Stepwise (Conditional) Omnibus Tests of Model Coefficients

	Chi-square	df	Sig.
Step 1	Step	6.700	1
	Block	6.700	1
	Model	20.521	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	278.845 ^a	.091	.121

a. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	1.640	6	.950

Contingency Table for Hosmer and Lemeshow Test

	T2DM Status = 0		T2DM Status = 1		Total
	Observed	Expected	Observed	Expected	
Step 1	1	27	27.041	10	9.959
	2	11	12.384	8	6.616
	3	30	30.499	26	25.501
	4	8	6.932	5	6.068

5	18	16.720	19	20.280	37
6	9	8.334	14	14.666	23
7	4	5.410	15	13.590	19
8	3	2.680	9	9.320	12

Classification Table^a

Observed	T2DM Status	Predicted		Percentage Correct
		0	1	
Step 1	T2DM Status	0	76	34
		1	49	57
Overall Percentage				61.6

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs7903146		11.494	2	.003	
	rs7903146(1)	.820	.311	1	.008	2.270
	rs7903146(2)	1.701	.584	1	.004	5.479
	Sex(1)	-.372	.294	1.600	1	.206
	Age (1)	.866	.341	6.449	1	.011
	Constant	-.627	.325	3.727	1	.054

Variables in the Equation

			95% C.I. for EXP(B)	
			Lower	Upper
Step 1 ^a	rs7903146			
	rs7903146(1)		1.235	4.174
	rs7903146(2)		1.743	17.222
	Sex(1)		.387	1.227
	Age (1)		1.218	4.636
	Constant			

Model if Term Removed^a

Variable	Model Log Likelihood	Change in -2 Log Likelihood	df	Sig. of the Change
Step 1 Age	-142.779	6.714	1	.010

a. Based on conditional parameter estimates

Logistic regression analysis to test association between rs12255372 and T2DM

Block 0: Beginning Block

		Classification Table ^{a,b}		Predicted T2DM Status 0 1 Percentage Correct	
Observed		0	1		
Step 0	T2DM Status	0	110	0	100.0
		1	106	0	.0
Overall Percentage					50.9

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-.037	.136	.074	1	.786

Variables not in the Equation

	Variables	Score	df	Sig.
Step 0	rs12255372	6.240	2	.044
	rs12255372(1)	5.193	1	.023
	rs12255372(2)	.436	1	.509
Overall Statistics		6.240	2	.044

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	6.276	2	.043
	Block	6.276	2	.043
	Model	6.276	2	.043

Model Summary

Step	-2 Log likelihood	Cox & Snell R	Nagelkerke R
		Square	Square
1	293.090 ^a	.029	.038

a. Estimation terminated at iteration number 3 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	.000	1	1.000

Contingency Table for Hosmer and Lemeshow Test

		T2DM Status = 0		T2DM Status = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	81	81.000	61	61.000	142
	2	5	5.000	7	7.000	12
	3	24	24.000	38	38.000	62

Classification Table^a

	Observed	Predicted T2DM Status		Percentage Correct
		0	1	
Step 1	T2DM Status	0	81	29
		1	61	45
	Overall Percentage			58.3

a. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs12255372		6.148	2	.046	
	rs12255372(1)	.743	.311	1	.017	2.102
	rs12255372(2)	.620	.610	1	.309	1.859
	Constant	-.284	.170	1	.094	.753

Variables in the Equation

		95% C.I. for EXP(B)	
		Lower	Upper
Step 1 ^a	rs12255372		
	rs12255372(1)	1.143	3.868
	rs12255372(2)	.563	6.140
	Constant		

a. Variable(s) entered on step 1: rs12255372.

Block 2: Method = Enter

Omnibus Tests of Model Coefficients

	Chi-square	df	Sig.
Step 1	Step	1.562	1
	Block	1.562	1
	Model	7.838	.049

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	291.527 ^a	.036	.048

a. Estimation terminated at iteration number 3 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	.442	2	.802

Contingency Table for Hosmer and Lemeshow Test

	T2DM Status = 0		T2DM Status = 1		Total	
	Observed	Expected	Observed	Expected		
Step 1	1	57	55.888	36	37.112	93
	2	24	25.112	25	23.888	49
	3	16	17.112	24	22.888	40
	4	13	11.888	21	22.112	34

Classification Table^a

Observed	Predicted		Percentage Correct	
	T2DM Status 0	1		
Step 1	T2DM Status 0	81	29	73.6
	1	61	45	42.5
Overall Percentage			58.3	

a. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs12255372		5.560	2	.062	
	rs12255372(1)	.722	.312	1	.021	2.058
	rs12255372(2)	.508	.618	1	.411	1.663
	Sex(1)	-.359	.288	1	.212	.698
	Constant	-.050	.252	1	.843	.951

Variables in the Equation

		95% C.I. for EXP(B)	
		Lower	Upper
Step 1 ^a	rs12255372		
	rs12255372(1)	1.116	3.797
	rs12255372(2)	.495	5.581
	Sex(1)	.397	1.227
	Constant		

a. Variable(s) entered on step 1: Sex.

Block 3: Method = Forward Stepwise (Conditional)

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	5.639	1	.018
	Block	5.639	1	.018
	Model	13.477	4	.009

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	285.888 ^a	.060	.081

a. Estimation terminated at iteration number 3 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	9.794	4	.044

Contingency Table for Hosmer and Lemeshow Test

Step	1	T2DM Status = 0		T2DM Status = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	43	46.516	29	25.484	72
	2	19	20.575	18	16.425	37
	3	16	13.414	12	14.586	28
	4	14	9.544	7	11.456	21
	5	13	10.494	14	16.506	27
	6	5	9.456	26	21.544	31

Classification Table^a

Observed	T2DM Status	Predicted		Percentage Correct	
		T2DM Status			
		0	1		
Step 1	0	62	48	56.4	
	1	47	59	55.7	
Overall Percentage				56.0	

a. The cut value is .500

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	rs12255372		5.272	2	.072	
	rs12255372(1)	.694	.317	1	.028	2.002
	rs12255372(2)	.632	.625	1	.311	1.882
	Sex(1)	-.376	.292	1	.197	.686
	Age (1)	.784	.335	1	.019	2.191
	Constant	-.225	.265	1	.396	.798

Variables in the Equation

	B	95% C.I. for EXP(B)	
		Lower	Upper
Step 1 ^a	rs12255372		
	rs12255372(1)	1.077	3.724
	rs12255372(2)	.553	6.401

Sex(1)	.387	1.216
Age (1)	1.136	4.227
Constant		

a. Variable(s) entered on step 1: Age .

Model if Term Removed^a					
Variable	Model Log Likelihood	Change in -2 Log Likelihood	df	Sig. of the Change	
Step 1 Age	-145.767	5.645	1		.018

a. Based on conditional parameter estimates