

Supplementary material

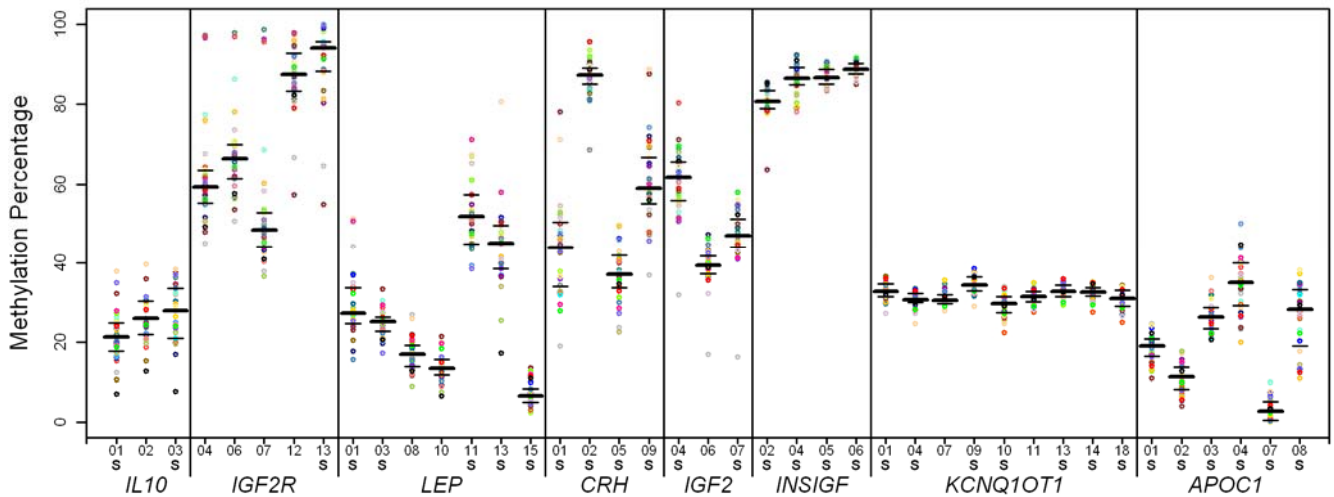


Figure S1: Methylation percentage, y-axis, at every CpG-unit, x-axis, for each individual, coloured dots. Measurement was done on DNA from the recent blood samples of the 34 individuals used in the experiments on temporal stability. The order of the loci is based on their chromosomal location, starting at the lowest designation. CpG-unit numbers are counted from the Forward primer onward. The name of each locus is given below the x-axis. The bold horizontal bar gives the median, the thin horizontal bars show the inter-quartile range for each CpG-unit. The vertical lines across the plot separate the loci. The corresponding CpG-sites of each CpG-unit are given in table S4C. Individually measured CpG-sites are marked with an “S” below the unit number.

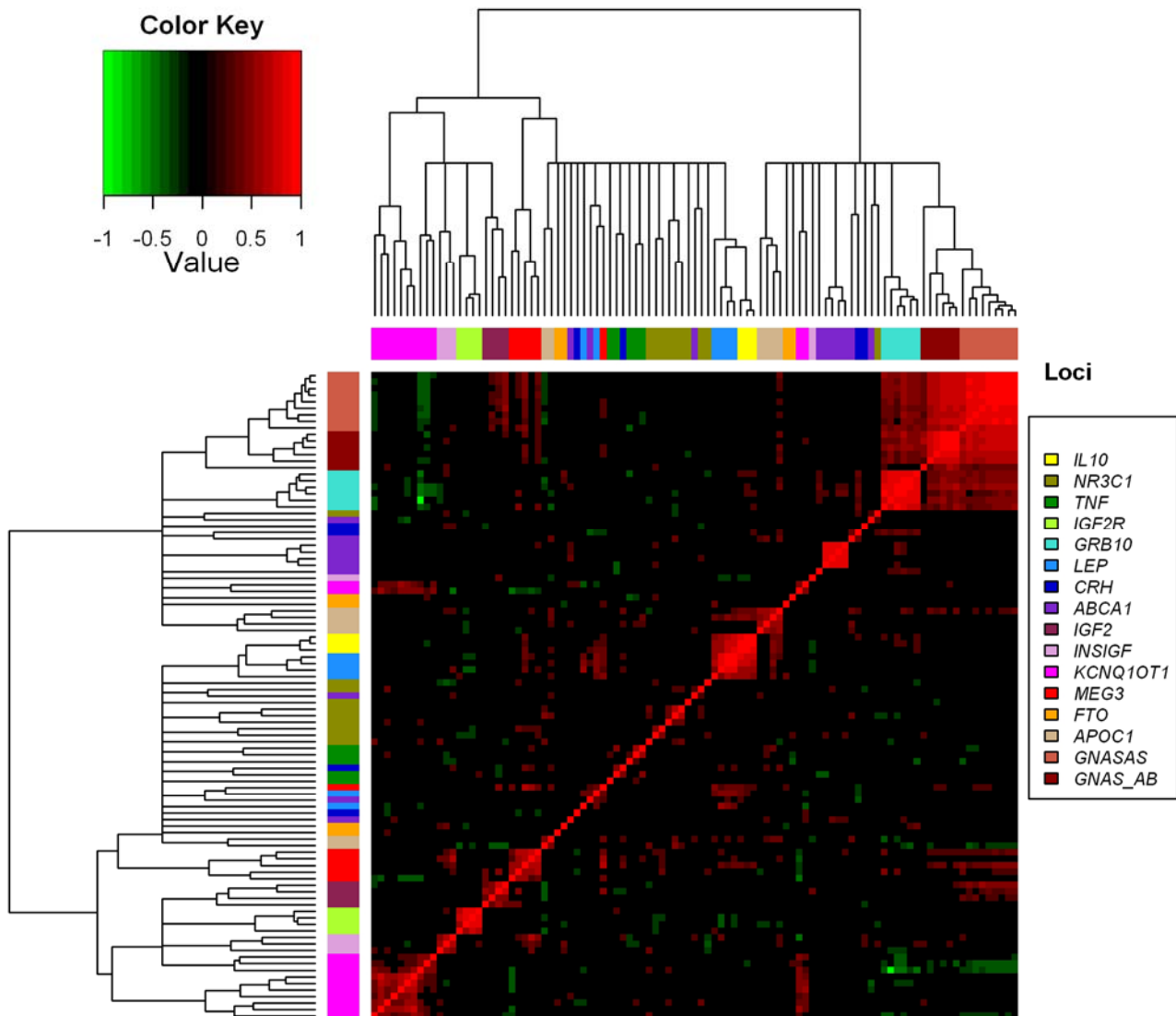


Figure S2: Heatmap depicting correlations between the methylation levels of all CpG-units of the 16 loci measured in the controls of the periconceptional group in the Dutch Hunger Winter Families study (P.I: Dr. L.H. Lumey, Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA). Correlations were computed on the data after variance stabilizing transformation. For reference the CpG-units are annotated by a colour, based on the locus, in the left and upper margin. The diagonal axis running from the lower left to the upper right corner is the line of symmetry where each CpG-unit hypothetically correlates with itself. Full correlation (1) is plotted as the brightest red shade, full inverse correlation (-1) is plotted as the brightest green shade, no correlation (0) is plotted as black. Non-significant correlations are depicted as no correlation. The complete clustering is based on the Euclidean distance.

Table S1: Association of neutrophil proportion with DNA methylation of the recent blood samples of 34 additional individuals from the NTR biobank

Locus	Variance explained	p-value of effect
<i>IL10</i>	27.9 %	$8.0 \cdot 10^{-08}$
<i>IGF2R</i>	1.2 %	0.312
<i>LEP</i>	1.8 %	$1.0 \cdot 10^{-04}$
<i>CRH</i>	0.0 %	0.993
<i>IGF2</i>	0.6 %	0.378
<i>INSIGF</i>	0.1 %	0.677
<i>KCNQ1OT1</i>	0.0 %	0.808
<i>APOC1</i>	0.1 %	0.763

Table S2: Comparison of DNA methylation in buccal cell samples from 34 individuals at two time points

Locus	Methylation mean (SD)		Difference mean (SD)	Spearman's rho (ρ)
	Old Buccal cells	New Buccal cells		
<i>IL10</i>	66.9% (13.6)	65.8% (18.4)	1.1% (17.0)	0.613
<i>IGF2R</i>	81.0% (12.9)	82.9% (11.7)	- 1.9% (9.0)	0.701
<i>LEP</i>	10.7% (7.4)	11.0% (7.8)	- 0.3% (7.3)	0.608
<i>CRH</i>	62.7% (21.0)	62.3% (21.5)	0.4% (9.2)	0.888
<i>IGF2</i>	32.5% (8.8)	32.3% (11.0)	0.2% (9.0)	0.686
<i>INSIGF</i>	84.6% (4.4)	84.4% (4.7)	0.2% (3.9)	0.543
<i>KCNQ1OT1</i>	35.8% (3.0)	34.9% (3.0)	0.9% (4.0)	0.097
<i>APOC1</i>	9.4% (7.2)	10.8% (9.7)	- 1.4% (6.1)	0.836

Table S3: Characteristics of 30 individuals, selected from the NTR biobank, studied

Male, no. (%)	12	(40 %)
Female, no. (%)	18	(60 %)
Smoker, no. (%)	3	(10 %)
Ex-smoker, no. (%)	15	(50 %)
Non-smoker, no. (%)	12	(40 %)
Age at biobanking, mean (SD)	48	(16)
Waist circumference in cm, mean (SD)	98	(22)
LDL-cholesterol (in mmol/L) , mean (SD)	2.47	(1.63)
HDL-cholesterol (in mmol/L) , mean (SD)	1.51	(0.94)
Glucose (in mmol/L) , mean (SD)	9.10	(4.83)

Table S4: Age and longitudinal sampling of 34 individuals from the NTR biobank

Age at first sampling (blood)	Years to first follow-up (buccal swab)	Years to second follow-up (blood and buccal swab)
62	6	13
62	6	13
60	9	12
54	6	14
52	6	13
52	6	13
48	6	13
48	6	13
46	10	12
46	10	12
45	10	12
45	10	12
43	6	13
43	6	12
42	6	11
41	6	13
41	10	12
41	6	12
41	10	12
39	6	11
39	9	12
36	10	12
36	10	14
36	10	12
34	9	12
21	13	15
19	10	16
17	15	17
17	14	16
16	11	16
16	13	16
14	17	19
14	16	18
14	15	19

Table S5A: Primers used in bisulfite PCR

Locus	Forward primer	Reverse primer
<i>IL10</i>	TGATTGGTTGAATATGAATTTTTGTAT	CACCCCTCATTTTTACTTAAAAA
<i>NR3C1</i>	GATTTGGTTTTTTTTGGGG	TCCCTTCCCTAAAACCT
<i>TNF</i>	GGGTATTTTTGATGTTTGTGTGTT	CAATACTCATAATATCCTTTCCAAAAAA
<i>IGF2R</i>	AGGTAGAAAAAGGTTTTGGAAG	CAAATCTTAAAACTAACTAAAAACC
<i>GRB10</i>	GGAATTTTAGGATTAATTTATGTGA	AACTTCCAAAAAAAACCTCTCC
<i>LEP</i>	GTTTTTGAGGGATATTAAGGATTT	CTACCAAAAAAACCAACAAAAAAA
<i>CRH</i>	TGGTTGTTGTTTTTTGGTAGG	AATTTCTCCACTCCAAAACCTAAA
<i>ABCA1</i>	ATTTTATTGGTGTTTTTGGTTGT	ATCAAAACCTATACTCTCCCTCCTC
<i>IGF2</i>	TGGATAGGAGATTGAGGAGAAA	AAACCCCAACAAAAACCACT
<i>INSIGF</i>	GTTTTGAGGAAGAGGTGTGA	ACCTAAAATCCAACCACCCTAA
<i>KCNQ1OT1</i>	TTTGGTAGGATTTTGTGAGGAGTTT	CTCACACCCAACCAATACCTCATAC
<i>MEG3</i>	TTTTTTTTAATAGTATTTTGATTTTTG	AAATAATCCCCACACATACC
<i>FTO</i>	GTTTGTAAATTTAGTATTTTGGGAGGT	TTTATTTCCATTTATCCATTCTCAA
<i>APOC1</i>	GGAGGAGGGAGATTAATATTAATTTGT	ACCCCAAACCTATAACCACCTT
<i>GNASAS</i>	GTAATTTGTGGTATGAGGAAGAGTGA	TAAATAACCCAACTAAATCCCAACA
<i>GNAS A/B</i>	ATGATTTAATTAAGGTTTTAGGAAAGG	TAAAAATACAAAACCTCCCCTACTC
10-mer tag	AGGAAGAGAG + primer	
T7 tag		CAGTAATACGACTCACTATAGGGAGAAGGCT + primer

Table S5B: CpG-sites per fragment of the loci that were analyzed for variation

Locus	CpG-sites* analyzed
<i>IL10</i>	1, 2&3, 4
<i>NR3C1</i>	1&2, 4, 7&8, 9, 10&11, 12&13, 14, 15&16, 17-20, 31, 33&34
<i>TNF</i>	1-3, 5, 6, 9, 10, 11
<i>IGF2R</i>	4&5, 8-10, 11-13, 20&21
<i>GRB10</i>	1&2, 4-6, 7, 8, 17, 18-21, 22&23, 24, 25
<i>LEP</i>	1, 8, 16&17, 19-21, 22, 25, 27
<i>CRH</i>	1, 2, 5, 9, 10
<i>ABCA1</i>	1, 3&4, 6-9, 15&16, 17&18, 19-21, 24, 25
<i>IGF2</i>	3, 4, 6&7, 8
<i>INSIGF</i>	2, 4, 5, 6
<i>KCNQ1OT1</i>	1, 6, 8&9, 10-12, 15, 16, 17&18, 20, 21, 25
<i>MEG3</i>	2, 3, 4, 8&9, 10&11
<i>FTO</i>	2&3, 7, 8&9, 10&11, 14, 17, 19
<i>APOC1</i>	1, 2, 3, 4, 10, 11
<i>GNASAS</i>	1&2, 3&4, 6, 7, 8&9, 10-12, 13&14, 15, 17-19
<i>GNAS A/B</i>	1, 3&4, 7, 8, 13-15, 16-19

* CpG-site number is counted from the forward primer onward

Table S5C: CpG-sites per fragment of the loci that were analyzed for stability

Locus	CpG-sites* analyzed
<i>IL10</i>	1, 2&3, 4
<i>IGF2R</i>	4&5, 8-10, 11-13, 20&21, 22 [†]
<i>LEP</i>	1, 8, 16&17, 19-21, 22 [†] , 25, 27
<i>CRH</i>	1 [†] , 2, 5, 9
<i>IGF2</i>	4, 6&7, 8
<i>INSIGF</i>	2, 4, 5, 6
<i>KCNQ1OT1</i>	1, 6, 10-12, 15, 16, 17&18, 20, 21, 25
<i>APOC1</i>	1, 2, 3, 4, 10, 11

* CpG-site number is counted from the forward primer onward

[†] CpG-site measurement met the quality criteria only in the recent blood samples

Table S6: All CpG-units of the 16 loci

Locus_CpG-unit*	CpG-sites*	Reason for removal prior to quality control
<i>IL10</i> _01	CpG_1	
<i>IL10</i> _02	CpG_2&3	
<i>IL10</i> _03	CpG_4	
<i>NR3C1</i> _01	CpG_1&2	
<i>NR3C1</i> _02	CpG_3	
<i>NR3C1</i> _03	CpG_4	
<i>NR3C1</i> _04	CpG_5&6	
<i>NR3C1</i> _05	CpG_7&8	
<i>NR3C1</i> _06	CpG_9	
<i>NR3C1</i> _07	CpG_10&11	
<i>NR3C1</i> _08	CpG_12&13	
<i>NR3C1</i> _09	CpG_14	
<i>NR3C1</i> _10	CpG_15&16	
<i>NR3C1</i> _11	CpG_17-20	
<i>NR3C1</i> _12	CpG_21	Mass overlap with unit 14
<i>NR3C1</i> _13	CpG_22-28	
<i>NR3C1</i> _14	CpG_29	Mass overlap with unit 12
<i>NR3C1</i> _15	CpG_30	
<i>NR3C1</i> _16	CpG_31	
<i>NR3C1</i> _17	CpG_32	
<i>NR3C1</i> _18	CpG_33&34	
<i>NR3C1</i> _19	CpG_35-41	High Mass
<i>NR3C1</i> _20	CpG_42	rs5871844 and rs34027900
<i>TNF</i> _01	CpG_1-3	
<i>TNF</i> _02	CpG_4	
<i>TNF</i> _03	CpG_5	
<i>TNF</i> _04	CpG_6	
<i>TNF</i> _05	CpG_7&8	
<i>TNF</i> _06	CpG_9	
<i>TNF</i> _07	CpG_10	
<i>TNF</i> _08	CpG_11	
<i>IGF2R</i> _01	CpG_1	Mass overlap with unit 8
<i>IGF2R</i> _02	CpG_2	Mass overlap with unit 3
<i>IGF2R</i> _03	CpG_3	Mass overlap with unit 2
<i>IGF2R</i> _04	CpG_4&5	
<i>IGF2R</i> _05	CpG_6&7	Mass overlap with unit 11
<i>IGF2R</i> _06	CpG_8-10	
<i>IGF2R</i> _07	CpG_11-13	
<i>IGF2R</i> _08	CpG_14	Mass overlap with unit 1
<i>IGF2R</i> _09	CpG_15&16	Mass overlap with unit 10 and rs677882 and rs8191722
<i>IGF2R</i> _10	CpG_17	Mass overlap with unit 9
<i>IGF2R</i> _11	CpG_18&19	Mass overlap with unit 5 and rs8191721 and rs8191720
<i>IGF2R</i> _12	CpG_20&21	
<i>IGF2R</i> _13	CpG_22	

Table S6 (continued A): All CpG-units of the 16 loci

Locus_CpG-unit*	CpG-sites*	Reason for removal prior to quality control
<i>GRB10_01</i>	CpG_1&2	
<i>GRB10_02</i>	CpG_3	Mass overlap with unit 9
<i>GRB10_03</i>	CpG_4-6	
<i>GRB10_04</i>	CpG_7	
<i>GRB10_05</i>	CpG_8	
<i>GRB10_06</i>	CpG_9&10	Mass overlap with unit 8
<i>GRB10_07</i>	CpG_11	Low mass
<i>GRB10_08</i>	CpG_12	Mass overlap with unit 6
<i>GRB10_09</i>	CpG_13	Mass overlap with unit 2
<i>GRB10_10</i>	CpG_14&15	
<i>GRB10_11</i>	CpG_16	
<i>GRB10_12</i>	CpG_17	
<i>GRB10_13</i>	CpG_18-21	
<i>GRB10_14</i>	CpG_22&23	
<i>GRB10_15</i>	CpG_24	
<i>GRB10_16</i>	CpG_25	
<i>LEP_01</i>	CpG_1	
<i>LEP_02</i>	CpG_2-7	High Mass and rs791620
<i>LEP_03</i>	CpG_8	
<i>LEP_04</i>	CpG_9&10	Mass overlap with unit 9
<i>LEP_05</i>	CpG_11	Mass overlap with units 12 and 6
<i>LEP_06</i>	CpG_12&13	Mass overlap with units 5 and 12
<i>LEP_07</i>	CpG_14&15	Mass overlap with unit 14
<i>LEP_08</i>	CpG_16&17	
<i>LEP_09</i>	CpG_18	Mass overlap with unit 4
<i>LEP_10</i>	CpG_19-21	
<i>LEP_11</i>	CpG_22	
<i>LEP_12</i>	CpG_23&24	Mass overlap with units 5 and 6
<i>LEP_13</i>	CpG_25	
<i>LEP_14</i>	CpG_26	Mass overlap with unit 7
<i>LEP_15</i>	CpG_27	
<i>LEP_16</i>	CpG_28	
<i>LEP_17</i>	CpG_29	rs2167270
<i>LEP_18</i>	CpG_30-32	High Mass
<i>CRH_01</i>	CpG_1	
<i>CRH_02</i>	CpG_2	
<i>CRH_03</i>	CpG_3	
<i>CRH_04</i>	CpG_4	
<i>CRH_05</i>	CpG_5	
<i>CRH_06</i>	CpG_6	Mass overlap with unit 7
<i>CRH_07</i>	CpG_7	Mass overlap with unit 6
<i>CRH_08</i>	CpG_8	
<i>CRH_09</i>	CpG_9	
<i>CRH_10</i>	CpG_10	

Table S6 (continued B): All CpG-units of the 16 loci

Locus_CpG-unit*	CpG-sites*	Reason for removal prior to quality control
<i>ABCA1</i> _01	CpG_1	
<i>ABCA1</i> _02	CpG_2	
<i>ABCA1</i> _03	CpG_3&4	
<i>ABCA1</i> _04	CpG_5	
<i>ABCA1</i> _05	CpG_6-9	
<i>ABCA1</i> _06	CpG_10-13	rs2246298
<i>ABCA1</i> _07	CpG_14	
<i>ABCA1</i> _08	CpG_15&16	
<i>ABCA1</i> _09	CpG_17&18	
<i>ABCA1</i> _10	CpG_19-21	
<i>ABCA1</i> _11	CpG_22&23	rs13306071
<i>ABCA1</i> _12	CpG_24	
<i>ABCA1</i> _13	CpG_25	
<i>ABCA1</i> _14	CpG_26&27	rs2740483
<i>IGF2</i> _01	CpG_1	rs3741208 and rs17883577
<i>IGF2</i> _02	CpG_2	rs3741209
<i>IGF2</i> _03	CpG_3	
<i>IGF2</i> _04	CpG_4	
<i>IGF2</i> _05	CpG_5	rs4930041
<i>IGF2</i> _06	CpG_6&7	
<i>IGF2</i> _07	CpG_8	
<i>INSIGF</i> _01	CpG_1	Low mass
<i>INSIGF</i> _02	CpG_2	
<i>INSIGF</i> _03	CpG_3	
<i>INSIGF</i> _04	CpG_4	
<i>INSIGF</i> _05	CpG_5	
<i>INSIGF</i> _06	CpG_6	
<i>KCNQ1OT1</i> _01	CpG_1	
<i>KCNQ1OT1</i> _02	CpG_2	
<i>KCNQ1OT1</i> _03	CpG_3-5	Mass overlap with unit 8
<i>KCNQ1OT1</i> _04	CpG_6	
<i>KCNQ1OT1</i> _05	CpG_7	Mass overlap with units 15 and 19
<i>KCNQ1OT1</i> _06	CpG_8&9	
<i>KCNQ1OT1</i> _07	CpG_10-12	
<i>KCNQ1OT1</i> _08	CpG_13&14	Mass overlap with unit 3
<i>KCNQ1OT1</i> _09	CpG_15	
<i>KCNQ1OT1</i> _10	CpG_16	
<i>KCNQ1OT1</i> _11	CpG_17&18	
<i>KCNQ1OT1</i> _12	CpG_19	
<i>KCNQ1OT1</i> _13	CpG_20	
<i>KCNQ1OT1</i> _14	CpG_21	
<i>KCNQ1OT1</i> _15	CpG_22	Mass overlap with units 5 and 19
<i>KCNQ1OT1</i> _16	CpG_23	rs7940500
<i>KCNQ1OT1</i> _17	CpG_24	rs379976
<i>KCNQ1OT1</i> _18	CpG_25	
<i>KCNQ1OT1</i> _19	CpG_26&27	Mass overlap with units 5 and 15

Table S6 (continued C): All CpG-units of the 16 loci

Locus_CpG-unit*	CpG-sites*	Reason for removal prior to quality control
<i>MEG3_01</i>	CpG_1	
<i>MEG3_02</i>	CpG_2	
<i>MEG3_03</i>	CpG_3	
<i>MEG3_04</i>	CpG_4	
<i>MEG3_05</i>	CpG_5	Mass overlap with unit 6
<i>MEG3_06</i>	CpG_6	Mass overlap with unit 5
<i>MEG3_07</i>	CpG_7	
<i>MEG3_08</i>	CpG_8&9	
<i>MEG3_09</i>	CpG_10&11	
<i>MEG3_10</i>	CpG_12-14	High Mass
<i>FTO_01</i>	CpG_1	Mass overlap with Unit 5
<i>FTO_02</i>	CpG_2&3	
<i>FTO_03</i>	CpG_4	Low mass
<i>FTO_04</i>	CpG_5	
<i>FTO_05</i>	CpG_6	Mass overlap with Unit 1
<i>FTO_06</i>	CpG_7	
<i>FTO_07</i>	CpG_8&9	
<i>FTO_08</i>	CpG_10&11	
<i>FTO_09</i>	CpG_12	Mass overlap with Unit 17
<i>FTO_10</i>	CpG_13	
<i>FTO_11</i>	CpG_14	
<i>FTO_12</i>	CpG_15	
<i>FTO_13</i>	CpG_16	
<i>FTO_14</i>	CpG_17	
<i>FTO_15</i>	CpG_18	
<i>FTO_16</i>	CpG_19	
<i>FTO_17</i>	CpG_20	Mass overlap with Unit 9
<i>APOCI_01</i>	CpG_1	
<i>APOCI_02</i>	CpG_2	
<i>APOCI_03</i>	CpG_3	
<i>APOCI_04</i>	CpG_4	
<i>APOCI_05</i>	CpG_5&6	rs402204
<i>APOCI_06</i>	CpG_7-9	High Mass and rs5111
<i>APOCI_07</i>	CpG_10	
<i>APOCI_08</i>	CpG_11	
<i>GNASAS_01</i>	CpG_1&2	
<i>GNASAS_02</i>	CpG_3&4	
<i>GNASAS_03</i>	CpG_5	
<i>GNASAS_04</i>	CpG_6	
<i>GNASAS_05</i>	CpG_7	
<i>GNASAS_06</i>	CpG_8&9	
<i>GNASAS_07</i>	CpG_10-12	
<i>GNASAS_08</i>	CpG_13&14	
<i>GNASAS_09</i>	CpG_15	
<i>GNASAS_10</i>	CpG_16	rs45596642
<i>GNASAS_11</i>	CpG_17-19	

Table S6 (continued D): All CpG-units of the 16 loci

Locus_CpG-unit*	CpG-sites*	Reason for removal prior to quality control
<i>GNAS A/B_01</i>	CpG_1	
<i>GNAS A/B_02</i>	CpG_2	Mass overlap with unit 4
<i>GNAS A/B_03</i>	CpG_3&4	
<i>GNAS A/B_04</i>	CpG_5&6	Mass overlap with unit 2
<i>GNAS A/B_05</i>	CpG_7	
<i>GNAS A/B_06</i>	CpG_8	
<i>GNAS A/B_07</i>	CpG_9&10	
<i>GNAS A/B_08</i>	CpG_11	Low mass
<i>GNAS A/B_09</i>	CpG_12	
<i>GNAS A/B_10</i>	CpG_13-15	
<i>GNAS A/B_11</i>	CpG_16-19	
Totals		
# of amplicons		16
total # units		191
# units outside detection range		9
# units with equal or overlapping mass		36
# units with potential SNP		12
Total # CpG-units removed		87

* CpG-unit and CpG-site numbers are counted from the forward primer onward