

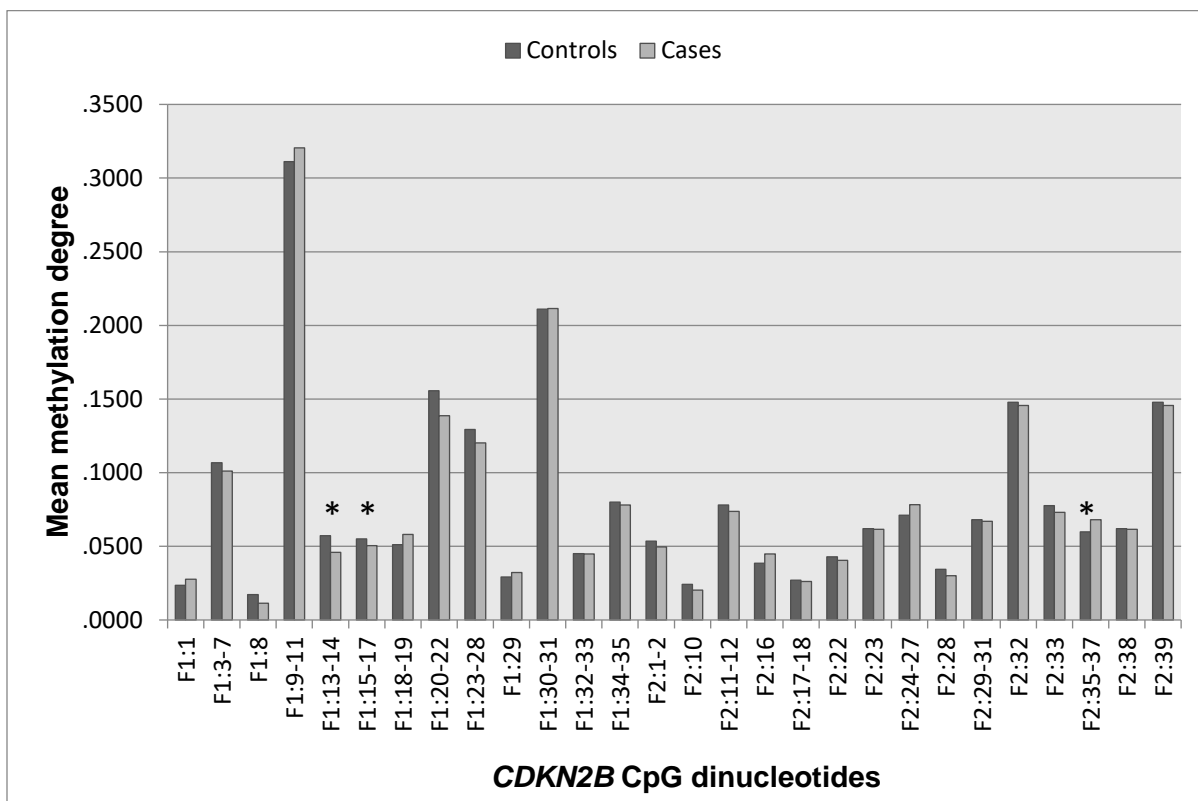
<i>CDKN2B</i> Assay ID	gDNA sequence ANNOTATED
Fragment 1	GAGAACAAGGGCATGCCAGTGGGGGCG ¹ GCAGCG ² ATGAGGGTCTGGCCAGCG ³ CCG ⁴ CG ⁵ GCG ⁶ CG ⁷ GGGACTAGTGGAGAAGGTGCG ⁸ ACAGCTCCTG GAAGCCCG ⁹ GCG ¹⁰ CG ¹¹ GATCCCAA ¹² CG ¹² GAG[TCAACCG ¹³ TTT ¹⁴ CG ¹⁴ GGAGGCG ¹⁵ CG ¹⁶ CG ¹⁷ ATCCA]GGTAGCTGGGGCCCCAGGGCCTCG ¹⁸ CG ¹⁹ GCAGGG GGCG ²⁰ CG ²¹ CG ²² AACG ²³ CG ²⁴ GGGCG ²⁵ CG ²⁶ GCCTCG ²⁷ GCG ²⁸ GATCG ²⁹ GGGCTGGAACCTAGATCG ³⁰ CG ³¹ ATGTAGATTTGTACAGGAGTCTCG ³² TTGG CG ³³ GAGGTGTGCATTCCA ³⁴ CG ³⁴ CG ³⁵ TAAACAGGCTTTTACCCAGCAAAAATCCTAAAGAGAGACATTGAAAAACCCACTGTTTAAGCTTTTTTTAGTGTT TTTGTCTGCCATCTCATGA
Fragment 2	TCCTAGCATCTTTGGCAGGCTTCCC ¹ CG ¹ CCCTCG ² TGACCG ³ CG ⁴ T ⁵ CG ⁵ GCCCG ⁶ GGCCTGGCCTCCCG ⁷ GCG ⁸ ATCACAGCG ⁹ GACAGGGGGCG ¹⁰ GAGCCTAA GGGGGTGGGGAGAGCG ¹¹ CG ¹² GCCCCTGGCCAGCTGAAAA ¹³ CG ¹³ GAATCTTTGCG ¹⁴ GCTGGCTCCCCACTCTGCCAGAGCG ¹⁵ AGGCG ¹⁶ GGGCAGTGA GGACTCG ¹⁷ CG ¹⁸ ACG ¹⁹ CG ²⁰ TCCG ²¹ CACCCTGCG ²² GCCAGAGCG ²³ GCTTTGAGCTCG ²⁴ GCTGCG ²⁵ TCCG ²⁶ CG ²⁷ CTAGGCG ²⁸ CTTTTTCCAGAAGCAATCC AGGCG ²⁹ CG ³⁰ CCCG ³¹ CTGTTTCTT ³² GAGCG ³² CCAGGAAAAGCCCG ³³ GAG[CTAACCG ³⁴ ACCG ³⁵ GCCG ³⁶ CTCG ³⁷ GCCAC]TGCA ³⁸ CG ³⁸ GGGCCCAAGCG ³⁹ CA GAAGGACG ⁴⁰ ACG ⁴¹ GGAGGGTAATGAAGCTGAGCCAGG

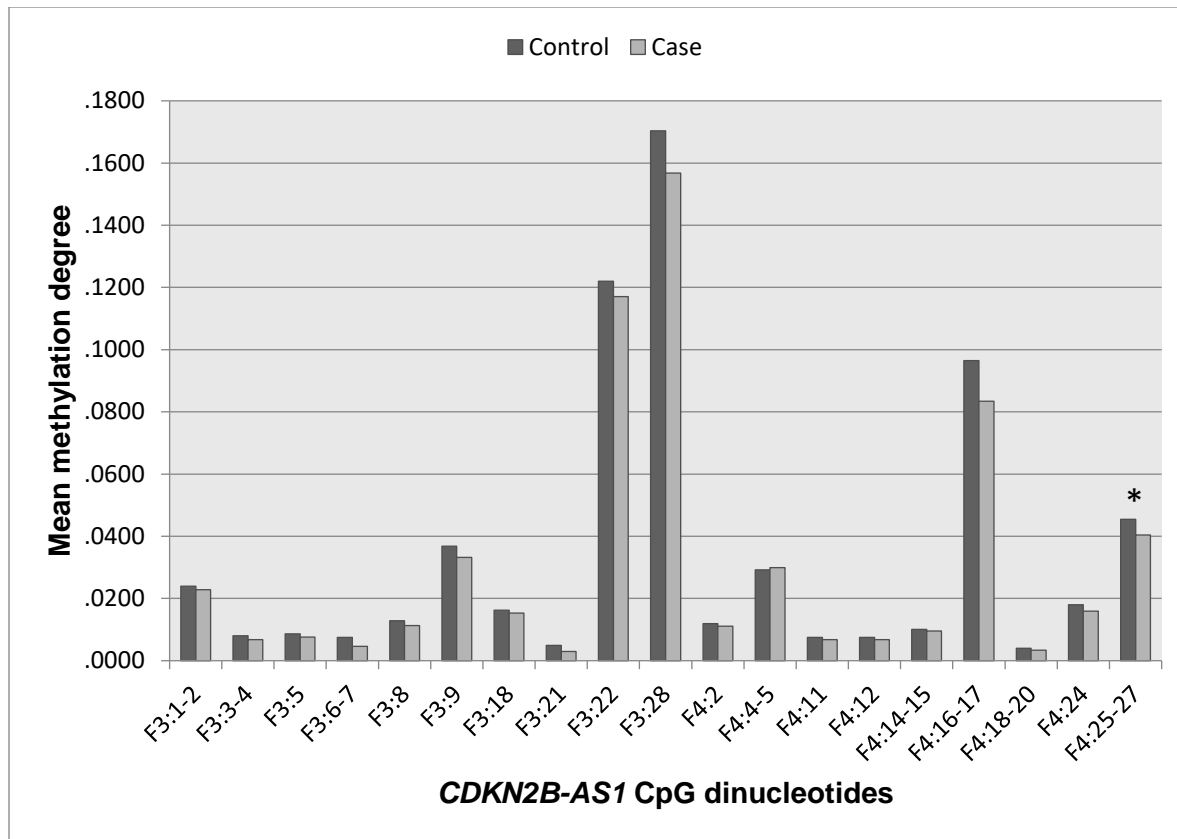
<i>CDKN2B-AS1</i> Assay ID	gDNA sequence ANNOTATED
Fragment 3	GAGTGGCCCTGCTCACCTCTGGTGCCAAAGGGCG ¹ GCG ² CAGCG ³ GCTGCG ⁴ AGCTCG ⁵ GCCCTGGAGGCG ⁶ GCG ⁷ AGAACATGGTGGCG ⁸ CAGGTTCTTGG TGACCCTCG ⁹ GATT ¹⁰ CG ¹⁰ GCG ¹¹ CG ¹² CG ¹³ TGCG ¹⁴ GCCCG ¹⁵ CGG ¹⁶ CG ¹⁷ AGTGAGGGTTTT ¹⁸ CG ¹⁸ TGGTTCACATCCCG ¹⁹ CG ²⁰ GCTCACCG ²¹ GGGAGTGGGC AGCG ²² CCAGGGGCG ²³ CCCG ²⁴ CGG ²⁵ CTGTGGCCCTCG ²⁶ TGCTGATGCTACTGAGGAGCCAGCG ²⁷ TCTAGGGCAGCAGCG ²⁸ CTTCTAGAAGACCAGGT AGGAAAGGCCCT
Fragment 4	TTTCCTGTCTCCAGCTGAAAAGGAGGAAGGGAGAGAGTCCAGAAAGGATCG ¹ GTGATGTGGAAGAAAAGGGGAGGAGGGGACATGGAGGGGGAGAC CG ² GAGAGAGAA ³ CG ³ TACG ⁴ CG ⁵ AGGAGTCAGGCG ⁶ GCG ⁷ GGATCAAGGGGAGT ⁸ CG ⁸ GGGTGTCTGGGCG ⁹ CG ¹⁰ GGGCAGAGCG ¹¹ TGGAGGCG ¹² GCAG CG ¹³ GCCAA ¹⁴ CG ¹⁴ GT ¹⁵ CG ¹⁵ CCAAGACAACCATTCTA ¹⁶ CG ¹⁶ CG ¹⁷ AGGACG ¹⁸ CG ¹⁹ GCG ²⁰ ACAGGAGGGGAGCG ²¹ GCCAGCAGGGGAGGGGAGCG ²² CG ²³ GGG GAAGAGGAAAGAGGAAGAAGCG ²⁴ CTCAGATGCTCG ²⁵ CG ²⁶ GCTGT ²⁷ CG ²⁷ TGAAGGTTAAAACCG ²⁸ AAAATAAAAATGGGCTAGACACAAAGGACTC

Supplementary Figure 1; Physical sequence of each fragment in *CDKN2B* (fragment 1 and fragment 2) and *CDKN2B-AS1* (fragment 3 and fragment 4) with the numbering of the CpG sites.

Supplementary Table 1: Primer sequences and number of CpG sites in each fragment covering the CpG islands in the promoters of the CDKN2B and CDKN2B-AS1 genes

Gene	Fragment	Forward primer	Reverse Primer	PCR product size (bp)	No. CpG sites	No. resolvable CpG sites
CDKN2B	1	GAGAATAAGGGTATGTTTAGTGGGG	TCATAAAATAACAAAACAAAACCA	383	35	28
	2	TTTTAGTATTTTTGGGTAGGTTTTTT	CCTAAACTCAACTTCATTACCCTCC	397	41	
CDKN2B-AS1	3	GAGTGGCCCTGCTCACCTCTGGTGC	AGGGCCTTTCCTACCTGGTCTTCTA	282	28	19
	4	TTTCCTGTCTCCCAGCTGGAAAGGA	GAGTCCTTTGTGTCTAGCCCATTTTT	358	28	





Supplementary figure 2: Mean degree of methylation by CpG dinucleotide in (A) *CDKN2B* and (B) *CDKN2B-AS1* for NTG cases and controls. * indicates significant CpG sites, further details of the significant CpG sites are presented in Table 2.

Supplementary table 2: Association of methylation of CpG dinucleotides in *CDKN2B* and *CDKN2B-AS1* promoters with NTG.

CDKN2B					CDKN2B-AS1				
Fragment:CpG site(s)	Glaucoma status	Number of participants	Mean Rank	P-Value	Fragment:CpG site(s)	Glaucoma status	Number of participants	Mean Rank	P-Value
F1:1	Controls	188	180.98	0.924	F3:1-2	Controls	199	193.12	0.321
	Cases	172	179.98			Cases	176	182.21	
	Total	360				Total	375		
F1:3-7	Controls	184	183.55	0.388	F3:3-4	Controls	199	193.54	0.248
	Cases	173	174.16			Cases	176	181.74	
	Total	357				Total	375		
F1:8	Controls	146	151.61	0.090	F3:5	Controls	199	196.60	0.070
	Cases	145	140.35			Cases	176	178.27	
	Total	291				Total	375		
F1:9-11	Controls	187	171.67	0.078	F3:6-7	Controls	199	194.19	0.151
	Cases	174	191.03			Cases	176	181.00	
	Total	361				Total	375		
F1:13-14	Controls	187	197.16	0.001	F3:8	Controls	199	196.77	0.079
	Cases	173	162.49			Cases	176	178.08	
	Total	360				Total	375		
F1:15-17	Controls	168	176.08	0.012	F3:9	Controls	199	196.61	0.094
	Cases	158	150.12			Cases	176	178.26	
	Total	326				Total	375		
F1:18-19	Controls	187	174.06	0.187	F3:18	Controls	199	190.05	0.685
	Cases	174	188.46			Cases	176	185.68	
	Total	361				Total	375		
F1:20-22	Controls	188	187.76	0.165	F3:21	Controls	199	193.83	0.057
	Cases	172	172.57			Cases	176	181.41	
	Total	360				Total	375		
F1:23-28	Controls	181	186.40	0.093	F3:22	Controls	196	190.66	0.430

	Cases	173	168.18			Cases	176	181.86	
	Total	354				Total	372		
F1:29	Controls	188	176.52	0.342	F3:28	Controls	199	190.84	0.589
	Cases	174	186.88			Cases	176	184.79	
	Total	362				Total	375		
F1:30-31	Controls	182	174.80	0.892	F4:2	Controls	194	181.15	0.256
	Cases	168	176.26			Cases	176	190.30	
	Total	350				Total	370		
F1:32-33	Controls	188	181.77	0.960	F4:4-5	Controls	192	180.75	0.473
	Cases	174	181.21			Cases	176	188.59	
	Total	362				Total	368		
F1:34-35	Controls	174	171.16	0.674	F4:11	Controls	196	188.98	0.534
	Cases	163	166.70			Cases	176	183.73	
	Total	337				Total	372		
F2:1-2	Controls	190	186.92	0.400	F4:12	Controls	196	188.98	0.534
	Cases	174	177.68			Cases	176	183.73	
	Total	364				Total	372		
F2:10	Controls	179	175.64	0.480	F4:14-15	Controls	196	188.78	0.619
	Cases	165	169.09			Cases	176	183.96	
	Total	344				Total	372		
F2:11-12	Controls	191	189.77	0.164	F4:16-17	Controls	194	188.11	0.614
	Cases	173	174.47			Cases	176	182.62	
	Total	364				Total	370		
F2:16	Controls	187	173.61	0.324	F4:18-20	Controls	196	189.13	0.485
	Cases	169	183.91			Cases	176	183.57	
	Total	356				Total	372		
F2:17-18	Controls	192	184.82	0.799	F4:24	Controls	196	183.15	0.514
	Cases	174	182.04			Cases	176	190.23	
	Total	366				Total	372		
F2:22	Controls	192	191.16	0.143	F4:25-27	Controls	196	201.45	0.003

	Cases	174	175.05		Cases	176	169.85	
	Total	366			Total	372		
F2:23	Controls	192	182.58	0.860				
	Cases	174	184.51					
	Total	366						
F2:24-27	Controls	190	178.10	0.514				
	Cases	172	185.25					
	Total	362						
F2:28	Controls	192	188.79	0.264				
	Cases	173	176.57					
	Total	365						
F2:29-31	Controls	190	185.14	0.483				
	Cases	172	177.48					
	Total	362						
F2:32	Controls	159	158.18	0.815				
	Cases	154	155.79					
	Total	313						
F2:33	Controls	189	184.02	0.498				
	Cases	171	176.61					
	Total	360						
F2:35-37	Controls	192	171.75	0.031				
	Cases	173	195.49					
	Total	365						
F2:38	Controls	192	182.58	0.860				
	Cases	174	184.51					
	Total	366						
F2:39	Controls	159	158.18	0.815				
	Cases	154	155.79					
	Total	313						

Supplementary table 3: Association of methylation status of the CpG sites in *CDKN2B* and *CDKN2B-AS1* promoters with NTG, stratified by sex.

CDKN2B							
		Female			Male		
Fragment: CpG site(s)	Glaucoma status	Number of participants	Mean Rank	Mann-Whitney U	Number of participants	Mean Rank	Mann-Whitney U
F1:1	Controls	93	88.53	0.54	83	77.69	0.803
	Cases	79	84.11		73	79.42	
	Total	172			156		
F1:3-7	Controls	91	83.14	0.5	81	81.93	0.252
	Cases	79	88.22		74	73.70	
	Total	170			155		
F1:8	Controls	72	72.28	0.43	66	65.97	0.144
	Cases	68	68.61		59	59.68	
	Total	140			125		
F1:9-11	Controls	93	82.06	0.161	82	76.82	0.625
	Cases	80	92.74		74	80.36	
	Total	173			156		
F1:13-14	Controls	93	95.99	0.006	83	85.55	0.054
	Cases	79	75.33		74	71.65	
	Total	172			157		
F1:15-17	Controls	87	83.26	0.323	71	77.28	0.011
	Cases	72	76.06		66	60.09	
	Total	159			137		
F1:18-19	Controls	93	78.83	0.02	83	74.73	0.21
	Cases	80	96.50		74	83.79	
	Total	173			157		
F1:20-22	Controls	93	89.81	0.342	83	79.02	0.877
	Cases	79	82.60		73	77.90	
	Total	172			156		
F1:23-28	Controls	89	87.19	0.539	81	77.75	0.942
	Cases	80	82.57		73	77.23	
	Total	169			154		

F1:29	Controls	93	85.86	0.744	83	77.70	0.703
	Cases	80	88.33		74	80.45	
	Total	173			157		
F1:30-31	Controls	93	82.16	0.267	77	69.49	0.177
	Cases	78	90.58		70	78.96	
	Total	171			147		
F1:32-33	Controls	93	84.02	0.394	83	78.63	0.913
	Cases	80	90.47		74	79.42	
	Total	173			157		
F1:34-35	Controls	89	87.63	0.232	75	70.03	0.256
	Cases	77	78.72		69	75.19	
	Total	166			144		
F2:1-2	Controls	92	85.68	0.711	85	80.18	0.722
	Cases	81	88.49		72	77.60	
	Total	173			157		
F2:10	Controls	87	87.41	0.064	79	74.04	0.989
	Cases	76	75.80		68	73.96	
	Total	163			147		
F2:11-12	Controls	92	85.65	0.809	86	84.01	0.174
	Cases	80	87.48		72	74.12	
	Total	172			158		
F2:16	Controls	90	81.44	0.363	84	76.83	0.832
	Cases	78	88.03		70	78.30	
	Total	168			154		
F2:17-18	Controls	93	91.61	0.242	86	76.12	0.301
	Cases	81	82.78		72	83.53	
	Total	174			158		
F2:22	Controls	93	94.72	0.041	86	81.80	0.486
	Cases	81	79.22		72	76.75	
	Total	174			158		
F2:23	Controls	93	81.25	0.077	86	82.26	0.404
	Cases	81	94.68		72	76.21	
	Total	174			158		
F2:24-27	Controls	93	78.74	0.019	84	81.85	0.243

	Cases	80	96.61		71	73.44	
	Total	173			155		
F2:28	Controls	93	87.02	0.996	86	84.02	0.17
	Cases	80	86.98		72	74.10	
	Total	173			158		
F2:29-31	Controls	93	84.90	0.549	84	81.88	0.239
	Cases	80	89.44		71	73.41	
	Total	173			155		
F2:32	Controls	78	77.92	0.385	70	67.14	0.791
	Cases	71	71.79		65	68.92	
	Total	149			135		
F2:33	Controls	90	85.27	0.938	86	81.05	0.532
	Cases	79	84.69		71	76.52	
	Total	169			157		
F2:35-37	Controls	93	77.44	0.006	86	77.95	0.639
	Cases	80	98.12		72	81.35	
	Total	173			158		
F2:38	Controls	93	81.25	0.077	86	82.26	0.404
	Cases	81	94.68		72	76.21	
	Total	174			158		
F2:39	Controls	78	77.92	0.385	70	67.14	0.791
	Cases	71	71.79		65	68.92	
	Total	149			135		

CDKN2B-AS1							
		Female			Male		
Fragment: CpG site(s)	Glaucoma status	Number of participants	Mean Rank	Mann-Whitney U	Number of participants	Mean Rank	Mann-Whitney U
F3:1-2	Controls	103	104.65	0.351	96	88.22	0.832
	Cases	98	97.17		78	86.62	
	Total	201			174		
F3:3-4	Controls	103	108.17	0.05	96	85.96	0.622
	Cases	98	93.47		78	89.39	
	Total	201			174		
F3:5	Controls	103	105.10	0.264	96	91.90	0.14
	Cases	98	96.69		78	82.09	
	Total	201			174		
F3:6-7	Controls	103	104.60	0.25	96	89.90	0.414
	Cases	98	97.22		78	84.54	
	Total	201			174		
F3:8	Controls	103	108.72	0.042	96	89.04	0.636
	Cases	98	92.89		78	85.61	
	Total	201			174		
F3:9	Controls	103	105.97	0.205	96	90.96	0.304
	Cases	98	95.78		78	83.24	
	Total	201			174		
F3:18	Controls	103	103.13	0.58	96	87.63	0.97
	Cases	98	98.77		78	87.35	
	Total	201			174		
F3:21	Controls	103	101.54	0.795	96	92.54	0.022
	Cases	98	100.43		78	81.29	
	Total	201			174		
F3:22	Controls	101	103.00	0.455	95	87.49	0.886
	Cases	98	96.91		78	86.40	
	Total	199			173		
F3:28	Controls	103	100.95	0.99	96	90.58	0.37
	Cases	98	101.05		78	83.71	
	Total	201			174		
F4:2	Controls	100	95.47	0.165	94	86.10	0.872

	Cases	98	103.62		78	86.99	
	Total	198			172		
F4:4-5	Controls	100	97.30	0.579	92	83.96	0.653
	Cases	98	101.75		78	87.31	
	Total	198			170		
F4:11	Controls	102	101.96	0.642	94	87.73	0.624
	Cases	98	98.98		78	85.02	
	Total	200			172		
F4:12	Controls	102	101.96	0.642	94	87.73	0.624
	Cases	98	98.98		78	85.02	
	Total	200			172		
F4:14-15	Controls	102	104.02	0.309	94	85.37	0.708
	Cases	98	96.83		78	87.86	
	Total	200			172		
F4:16-17	Controls	100	101.23	0.661	94	86.97	0.888
	Cases	98	97.73		78	85.93	
	Total	198			172		
F4:18-20	Controls	102	99.91	0.827	94	89.44	0.257
	Cases	98	101.12		78	82.96	
	Total	200			172		
F4:24	Controls	102	93.92	0.092	94	89.60	0.354
	Cases	98	107.35		78	82.77	
	Total	200			172		
F4:25-27	Controls	102	112.08	0.003	94	89.44	0.375
	Cases	98	88.44		78	82.96	
	Total	200			172		

Supplementary table 4; Association between methylation of the associated CpG sites in *CDKN2B* and *CDKN2B-AS1* promoters and rs1063192 genotype.

CDKN2B					CDKN2B-AS1				
Fragment: CpG site(s)	rs1063192 genotype	N	Mean Rank	Mann-Whitney U	Fragment: CpG site(s)	rs1063192 genotype	N	Mean Rank	Mann-Whitney U
F1:1	Wild type	70	129.59	.087	F3:1-2	Wild type	77	129.01	0.655
	Homozygous	166	113.83			Homozygous	174	124.67	
	Total	236				Total	251		
F1:3-7	Wild type	69	119.48	.715	F3:3-4	Wild type	77	123.27	0.667
	Homozygous	164	115.96			Homozygous	174	127.21	
	Total	233				Total	251		
F1:8	Wild type	55	100.77	.197	F3:5	Wild type	77	129.35	0.592
	Homozygous	134	92.63			Homozygous	174	124.52	
	Total	189				Total	251		
F1:9-11	Wild type	70	130.76	.061	F3:6-7	Wild type	77	129.34	0.567
	Homozygous	165	112.58			Homozygous	174	124.52	
	Total	235				Total	251		
F1:13-14	Wild type	69	131.88	.042	F3:8	Wild type	77	135.39	0.149
	Homozygous	166	112.23			Homozygous	174	121.84	
	Total	235				Total	251		
F1:15-17	Wild type	67	126.84	.015	F3:9	Wild type	77	134.47	0.208
	Homozygous	154	104.11			Homozygous	174	122.25	
	Total	221				Total	251		
F1:18-19	Wild type	69	97.08	.002	F3:18	Wild type	77	122.26	0.572
	Homozygous	166	126.70			Homozygous	174	127.66	
	Total	235				Total	251		
F1:20-22	Wild type	70	117.40	.929	F3:21	Wild type	77	134.27	0.049
	Homozygous	165	118.25			Homozygous	174	122.34	
	Total	235				Total	251		
F1:23-28	Wild type	69	112.14	.432	F3:22	Wild type	77	127.86	0.674
	Homozygous	165	119.74			Homozygous	172	123.72	
	Total	234				Total	249		
F1:29	Wild type	70	116.57	.775	F3:28	Wild type	77	115.88	0.141

	Homozygous	166	119.31			Homozygous	174	130.48	
	Total	236				Total	251		
F1:30-31	Wild type	70	119.21	.630	F4:2	Wild type	73	126.73	0.607
	Homozygous	161	114.61			Homozygous	174	122.85	
	Total	231				Total	247		
F1:32-33	Wild type	70	119.99	.827	F4:4-5	Wild type	72	123.72	0.917
	Homozygous	166	117.87			Homozygous	173	122.70	
	Total	236				Total	245		
F1:34-35	Wild type	65	117.68	.182	F4:11	Wild type	75	126.17	0.823
	Homozygous	152	105.29			Homozygous	174	124.49	
	Total	217				Total	249		
F2:1-2	Wild type	74	109.89	.128	F4:12	Wild type	75	126.17	0.823
	Homozygous	165	124.54			Homozygous	174	124.49	
	Total	239				Total	249		
F2:10	Wild type	69	112.34	.908	F4:14-15	Wild type	75	134.17	0.145
	Homozygous	156	113.29			Homozygous	174	121.05	
	Total	225				Total	249		
F2:11-12	Wild type	74	114.86	.482	F4:16-17	Wild type	73	121.25	0.687
	Homozygous	164	121.59			Homozygous	174	125.16	
	Total	238				Total	247		
F2:16	Wild type	73	120.75	.607	F4:18-20	Wild type	75	132.43	0.16
	Homozygous	161	116.02			Homozygous	174	121.80	
	Total	234				Total	249		
F2:17-18	Wild type	74	115.61	.458	F4:24	Wild type	75	110.81	0.036
	Homozygous	166	122.68			Homozygous	174	131.11	
	Total	240				Total	249		
F2:22	Wild type	74	120.86	.956	F4:25-27	Wild type	75	136.63	0.085
	Homozygous	166	120.34			Homozygous	174	119.99	
	Total	240				Total	249		
F2:23	Wild type	74	122.19	.800					
	Homozygous	166	119.75						
	Total	240							
F2:24-27	Wild type	74	98.62	.001					
	Homozygous	165	129.59						

	Total	239		
F2:28	Wild type	74	123.03	.644
	Homozygous	165	118.64	
	Total	239		
F2:29-31	Wild type	74	106.24	.038
	Homozygous	165	126.17	
	Total	239		
F2:32	Wild type	55	110.17	.293
	Homozygous	150	100.37	
	Total	205		
F2:33	Wild type	73	135.05	.010
	Homozygous	162	110.32	
	Total	235		
F2:35-37	Wild type	74	97.34	.001
	Homozygous	165	130.16	
	Total	239		
F2:38	Wild type	74	122.19	.800
	Homozygous	166	119.75	
	Total	240		
F2:39	Wild type	55	110.17	.293
	Homozygous	150	100.37	
	Total	205		

Supplementary table 5; A comparison of the mean methylation values of CpG13 and CpG14 by massarray and pyrosequencing and in both female and male groups separately.

	Combined		Female		Male	
	Cases	Controls	Cases	Controls	Cases	Controls
Mass Array F13-14	4.59%	5.72%	4.33%	5.61%	4.88%	5.93%
Pyrosequencing 13	5.58%	5.69%	5.46%	5.84%	5.74%	5.52%
Pyrosequencing 14	9.91%	10.04%	9.83%	9.99%	10.00%	10.01%