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### Cover sheet

**Title**

DNA methylation at the 9p21 glaucoma susceptibility locus is associated with normal-tension glaucoma

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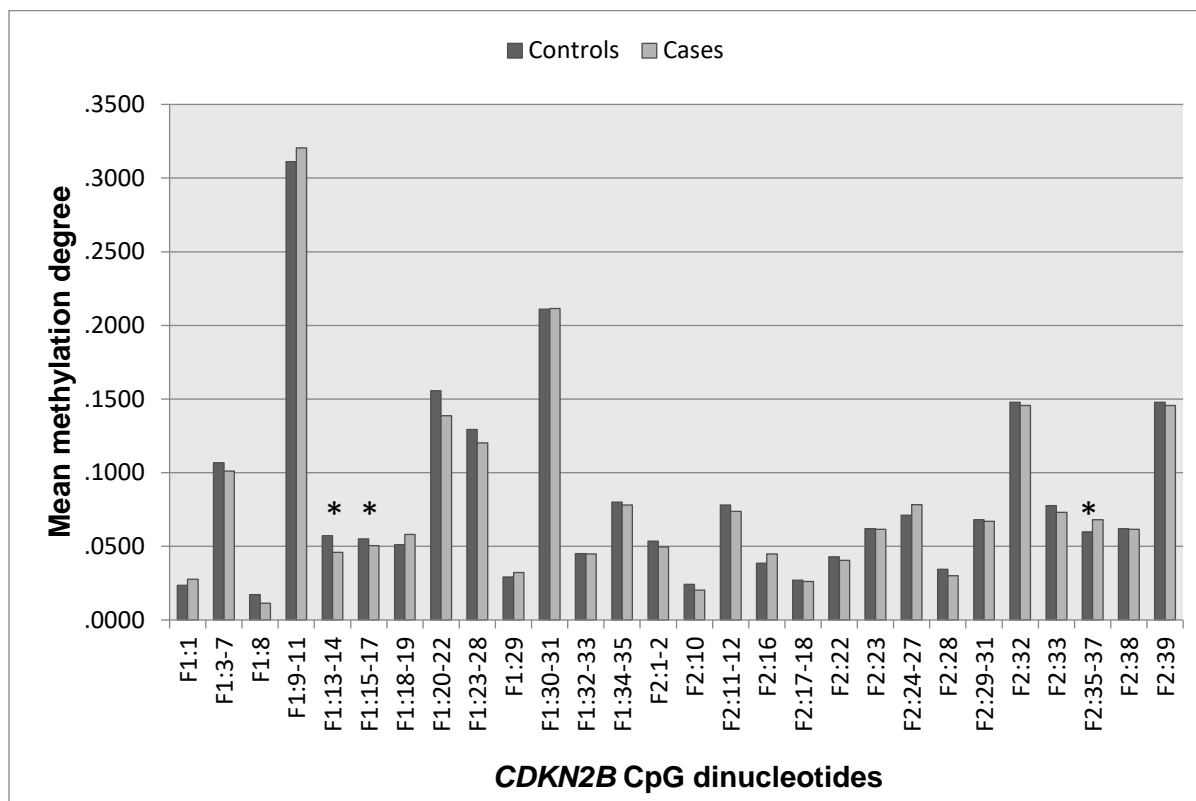
<i>CDKN2B</i> Assay ID	gDNA sequence ANNOTATED
Fragment 1	GAGAACAAAGGGCATGCCAGTGGGGGCG <sup>1</sup> GCAGCG <sup>2</sup> ATGAGGGTCTGGCCAGCG <sup>3</sup> CG <sup>4</sup> CG <sup>5</sup> GCG <sup>6</sup> CG <sup>7</sup> GGGACTAGTGGAGAAGGTGCG <sup>8</sup> ACAGCTCCTG GAAGCCCG <sup>9</sup> GCG <sup>10</sup> CG <sup>11</sup> GATCCCAACG <sup>12</sup> GAG[TCAACCG <sup>13</sup> TTT <sup>CG</sup> <sup>14</sup> GGAGGCG <sup>15</sup> CG <sup>16</sup> CG <sup>17</sup> ATCCA]GGTAGCTGGGGCCCCAGGGCCTCG <sup>18</sup> CG <sup>19</sup> GCAGGG GGCG <sup>20</sup> CG <sup>21</sup> CG <sup>22</sup> AACG <sup>23</sup> CG <sup>24</sup> GGGCG <sup>25</sup> CG <sup>26</sup> GCCTCG <sup>27</sup> GCG <sup>28</sup> GATCG <sup>29</sup> GGGCTGGAACCTAGATCG <sup>30</sup> CG <sup>31</sup> ATGTAGATTTGTACAGGAGTCTCG <sup>32</sup> TTGG CG <sup>33</sup> GAGGTGTGCATTCCA <sup>CG</sup> <sup>34</sup> CG <sup>35</sup> TAAACAGGCTTTTACCCAGCAAAAATCCTAAAGAGAGACATTGAAAAACCCACTGTTTAAGCTTTTTTTAGTGTT TTTGTCTGCCATCTCATGA
Fragment 2	TCCTAGCATCTTTGGGCAGGCTTCCC <sup>CG</sup> <sup>1</sup> CCCTCG <sup>2</sup> TGACCG <sup>3</sup> CG <sup>4</sup> T <sup>CG</sup> <sup>5</sup> GCCC <sup>CG</sup> <sup>6</sup> GGCCTGGCCTCCCG <sup>7</sup> GCG <sup>8</sup> ATCACAGCG <sup>9</sup> GACAGGGGGCG <sup>10</sup> GAGCCTAA GGGGGTGGGGAGACCG <sup>11</sup> CG <sup>12</sup> GCCCCTTGCCCAGCTGAAAA <sup>CG</sup> <sup>13</sup> GAATTCTTTGCCCG <sup>14</sup> GCTGGCTCCCCACTCTGCCAGAGCG <sup>15</sup> AGGCG <sup>16</sup> GGGCAGTGA GGA <sup>CTCG</sup> <sup>17</sup> CG <sup>18</sup> A <sup>CG</sup> <sup>19</sup> CG <sup>20</sup> TCCG <sup>21</sup> CACCCTGCG <sup>22</sup> GCCAGAGCG <sup>23</sup> GCTTTGAGCTCG <sup>24</sup> GCTGCG <sup>25</sup> TCCG <sup>26</sup> CG <sup>27</sup> CTAGGCG <sup>28</sup> CTTTTCCAGAAGCAATCC AGGCG <sup>29</sup> CG <sup>30</sup> CCCG <sup>31</sup> CTGTTCTTGAGCG <sup>32</sup> CCAGGAAAAGCCCG <sup>33</sup> GAG[CTAACCG <sup>34</sup> ACCG <sup>35</sup> GCCG <sup>36</sup> CTCG <sup>37</sup> GCCAC]TGCA <sup>CG</sup> <sup>38</sup> GGGCCCCAAGCG <sup>39</sup> CA GAAGGACG <sup>40</sup> ACG <sup>41</sup> GGAGGGTAATGAAGCTGAGCCCAGG

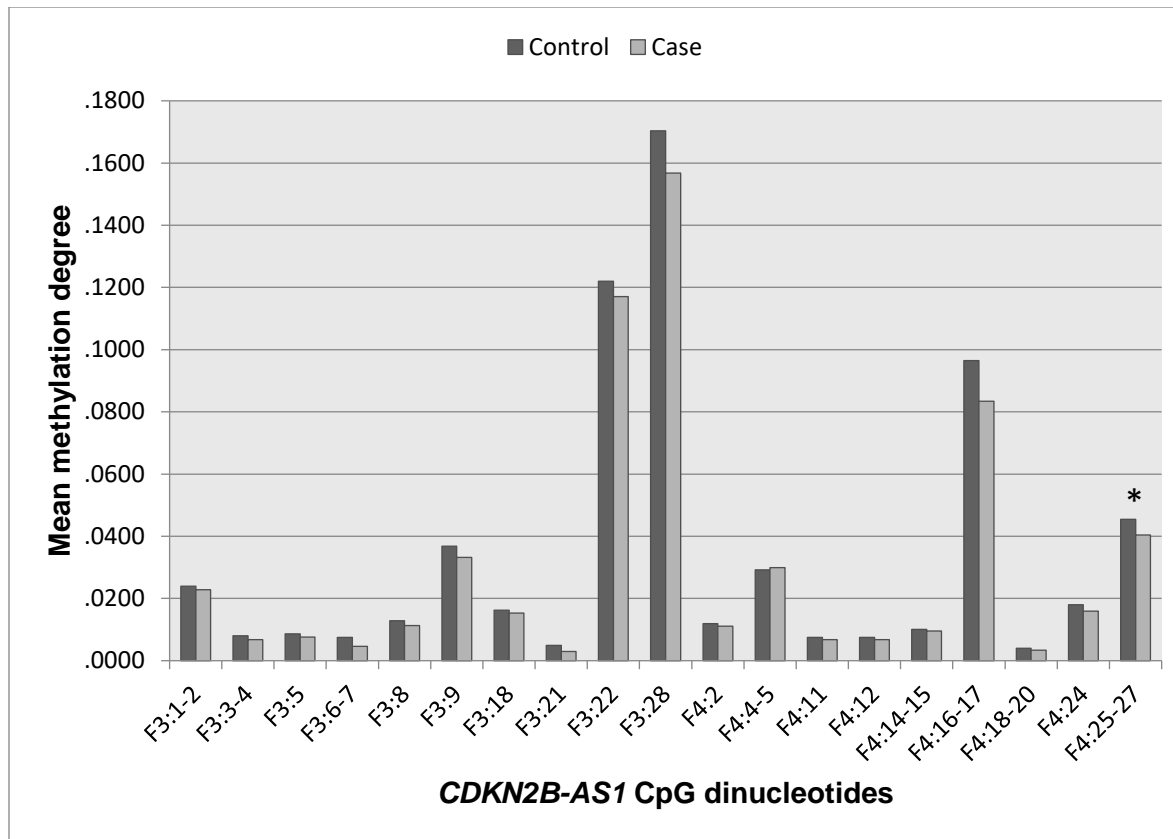
<i>CDKN2B-AS1</i> Assay ID	gDNA sequence ANNOTATED
Fragment 3	GAGTGGCCCTGCTCACCTCTGGTGCCAAAGGGCG <sup>1</sup> GCG <sup>2</sup> CAGCG <sup>3</sup> GCTGCCCG <sup>4</sup> AGCTCG <sup>5</sup> GCCCTGGAGGCG <sup>6</sup> GCG <sup>7</sup> AGAACATGGTGGCG <sup>8</sup> CAGGTTCTTGG TGACCTC <sup>CG</sup> <sup>9</sup> GATT <sup>CG</sup> <sup>10</sup> G <sup>CG</sup> <sup>11</sup> CG <sup>12</sup> CG <sup>13</sup> TGCG <sup>14</sup> GCCCG <sup>15</sup> CGG <sup>16</sup> CG <sup>17</sup> AGTGAGGGTTTT <sup>CG</sup> <sup>18</sup> TGGTTCACATCCCG <sup>19</sup> CG <sup>20</sup> GCTCACCG <sup>21</sup> GGGGAGTGGGC AGCG <sup>22</sup> CCAGGGGCG <sup>23</sup> CCCG <sup>24</sup> CG <sup>25</sup> CTGTGGCCCTCG <sup>26</sup> TGCTGATGCTACTGAGGAGCCAGCG <sup>27</sup> TCTAGGGCAGCAGCG <sup>28</sup> CTTCCTAGAAGACCAGGT AGGAAAGGCCCT
Fragment 4	TTTCCTGTCTCCAGCTGGAAAGGAGGAAGGGAGAGAGTCCAGAAAGGATCG <sup>1</sup> GTGATGTGGAAGAAAAGGGGAGGAGGGGACATGGAGGGGGAGAC CG <sup>2</sup> GAGAGAGAA <sup>CG</sup> <sup>3</sup> TACG <sup>4</sup> CG <sup>5</sup> AGGAGTCAGGCG <sup>6</sup> GCG <sup>7</sup> GGATCAAGGGGAGT <sup>CG</sup> <sup>8</sup> GGGTGTCTGGGCG <sup>9</sup> CG <sup>10</sup> GGGCAGAGCG <sup>11</sup> TGGAGGCG <sup>12</sup> GCAG CG <sup>13</sup> GCCAA <sup>CG</sup> <sup>14</sup> GT <sup>CG</sup> <sup>15</sup> CCAAGACAACCATTCTACG <sup>16</sup> CG <sup>17</sup> AGGACG <sup>18</sup> CG <sup>19</sup> GCG <sup>20</sup> ACAGGAGGGGAGCG <sup>21</sup> GCCAGCAGGGGAGGGGAGCG <sup>22</sup> CG <sup>23</sup> GGG GAAGAGGAAAGAGGAAGAAGCG <sup>24</sup> CTCAGATGCTCG <sup>25</sup> CG <sup>26</sup> GCTGT <sup>CG</sup> <sup>27</sup> TGAAGGTTAAACCG <sup>28</sup> 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	TTTLAGTATTTTGGGTAGGTTTTTT	CCTAAACTCAACTTCATTACCCTCC	397	41	
CDKN2B-AS1	3	GAGTGGCCCTGCTCACCTCTGGTGC	AGGGCCTTTCCTACCTGGTCTTCTA	282	28	19
	4	TTTCCTGTCTCCAGCTGGAAAGGA	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





**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%