Web Material

Neonatal genome-wide methylation patterns in relation to birthweight in the Norwegian Mother and Child Cohort

Web Figure 1 Legend

Manhattan plot of cord blood cytosine-guanine dinucleotides (CpG)-birthweight associations in the Norwegian Mother and Child cohort study (MoBa) using the Illumina Infinium HumanMethylation450 BeadChip. Models were adjusted for child sex, maternal plasma cotinine, parity, maternal age, dietary folate (not including supplements), asthma, gestational age at delivery, preeclampsia, season of birth and leukocyte cell type proportions. Bonferroni significance for 485,492 statistical tests is indicated by values above the red line ($p < 1.03 \times 10^{-7}$).

Web Figure 2 Legend

Associations of cytosine-guanine dinucleotides (CpG) with birthweight after additional adjustments**.** Negative $\log_{10}$ p-values of the individual CpG-birthweight associations are plotted after the inclusion of additional covariates (caffeinated beverage intake, maternal pre-pregnancy body mass index, and gestational weight gain up until the third trimester) in comparison to a model of the same sample size (n = 918) but without these additional covariates (Web Figure 2A, n = 918), and separately after adjustment for dietary folate (y-axis) and plasma folate (x-axis), along with the covariates in our main analysis (Web Figure 2B, n = 1046). There was no substantial difference in the estimated associations in either case.

Web Figure 3 Legend

Box plots of untransformed methylation beta value against the birthweight residual, after adjustment for child sex, maternal plasma cotinine, parity, maternal age, dietary folate not including supplements, asthma, gestational age at delivery, gestational age at delivery squared, preeclampsia, season of birth, and leukocyte cell type proportions for significant probes in Table 2 not already shown in Figures 1 and 2. Methylation beta values were divided into eight equally spaced intervals: A) cg08005122; B) cg20076442 C) cg25124943 D) cg00049440 E) cg17836177 F) cg00605777 G) cg23127323 H) cg25162533 I) cg17714703 J) cg08420923 K) cg23237276 L) cg05993265. Cg24693803 not pictured due to space.

Web Table 1. Associations of Selected CpGs and Birthweight in the Norwegian Mother and Child Birth Cohort from 1999 to 2008, using the Illumina Infinium HumanMethylation 450K Chip

CpG	Gene	Adjusted Mean Difference in Birthweight (SE)[a]	Bonferroni corrected p-value[b]
cg25953130	***ARID5B***	**-376.86 (53.93)**	**1.35 x 10^{-6}**
cg17714703	***UHRF1***	**322.68 (47.99)**	**8.64 x 10^{-6}**
cg02863179	***ARID5B***	**-340.46 (51.76)**	**2.32 x 10^{-5}**
cg20076442		**-304.93 (47.00)**	**4.22 x 10^{-5}**
cg17836177	***PEBP4***	**481.80 (78.73)**	**4.56 x 10^{-4}**
cg08420923	***ANKRD11***	**457.16 (75.05)**	**5.44 x 10^{-4}**
cg23494863	*STAR*	-516.91 (87.07)	1.41 x 10^{-3}
cg21058822	*MBOAT4*	-309.26 (52.21)	1.52 x 10^{-3}
cg23127323	***SPON2***	**257.23 (43.93)**	**2.31 x 10^{-3}**
cg00410926	*FAM166B*	-318.09 (55.59)	5.10 x 10^{-3}
cg15928780		-223.91 (39.68)	8.11 x 10^{-3}
cg27106909	*YPEL3*	511.68 (90.72)	8.25 x 10^{-3}
cg03861784		-395.35 (70.12)	8.35 x 10^{-3}
cg25162533		**371.26 (65.87)**	**8.40 x 10^{-3}**
cg00058449	*ZBTB12*	233.47 (41.88)	1.20 x 10^{-2}
cg20782117	*SLC25A13*	-302.97 (54.67)	1.46 x 10^{-2}
cg19620972	*GAL3ST3*	-224.33 (40.48)	1.46 x 10^{-2}
cg01800735	*NXN*	-224.96 (40.94)	1.90 x 10^{-2}
cg01400685	*FADS2*	-314.46 (58.28)	3.31 x 10^{-2}
cg17758673	*RGS12*	306.92 (57.01)	3.55 x 10^{-2}
cg05304729	*MNDA*	406.30 (75.71)	3.89 x 10^{-2}
cg09423126	*PEBP4*	357.02 (66.74)	4.28 x 10^{-2}

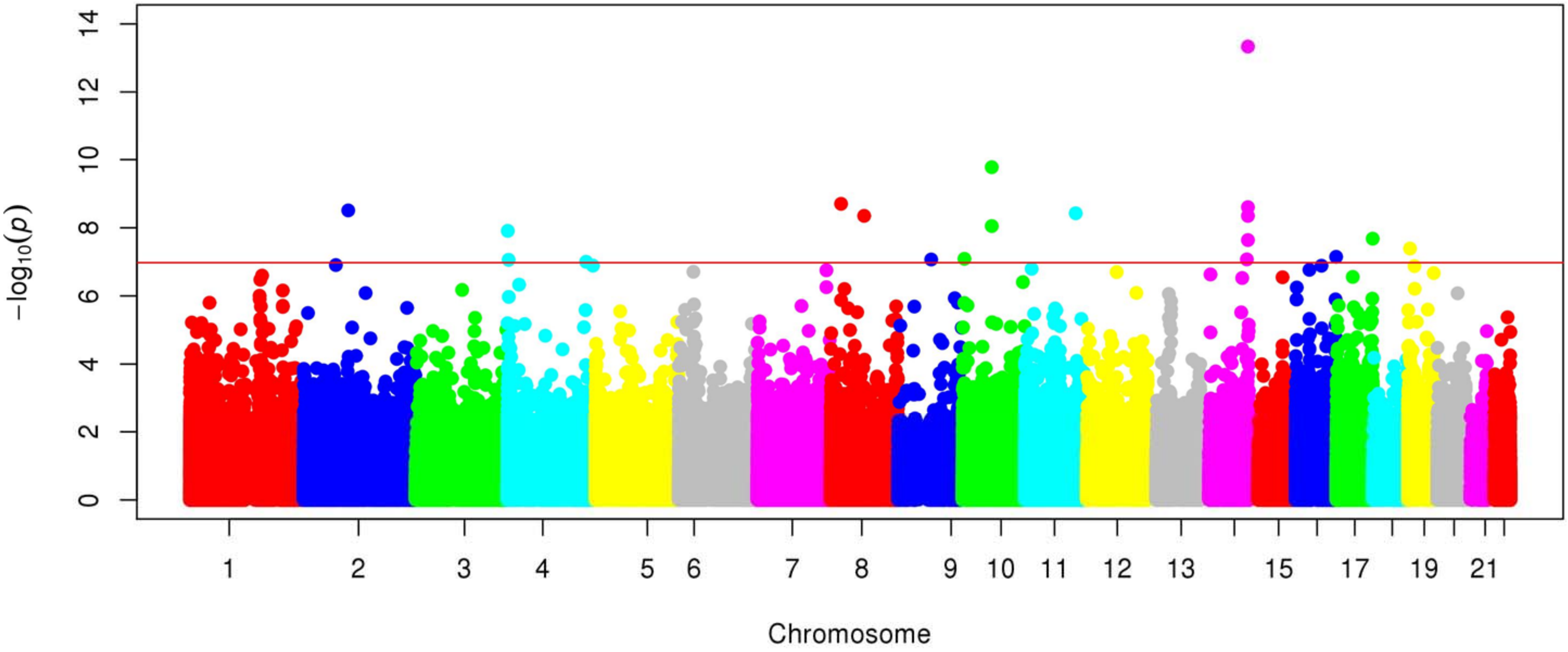
Ankyrin repeat domain 11 (*ANKRD11*), AT-rich interaction domain 5B (*ARID5B*), Cytosine-guanine dinucleotides (CpG), GNAS antisense RNA 1 (*GNASAS*), INS-IGF2 readthrough (*INSIGF*), Kruppel-like factor 9 (*KLF9*), Major facilitator superfamily domain containing 10 (*MFSD10*), Phosphatidylethanolamine-binding protein 4 (*PEBP4*), Sema domain, immunoglobulin domain, transmembrane domain and short cytoplasmic domain 4C (*SEMA4C*), Spondin 2 (*SPON2*), Standard Error (SE), Ubiquitin-like with PHD and ring finger domains 1 (*UHRF1*), X-ray repair complementing defective repair in Chinese hamster cells 3 (*XRCC3*), Yippee-like 3 (*YPEL3*)

Bolded CpGs are also significant after adjustment for cell type proportion (See Table 2).

[a] Adjusted mean difference in birthweight (SE) per logit increase in methylation fraction, including covariates for child sex, maternal plasma cotinine, parity, maternal age, dietary folate not including supplements, asthma, gestational age at delivery, gestational age at delivery squared, preeclampsia, and season of birth. Unadjusted for leukocyte cell type proportions.

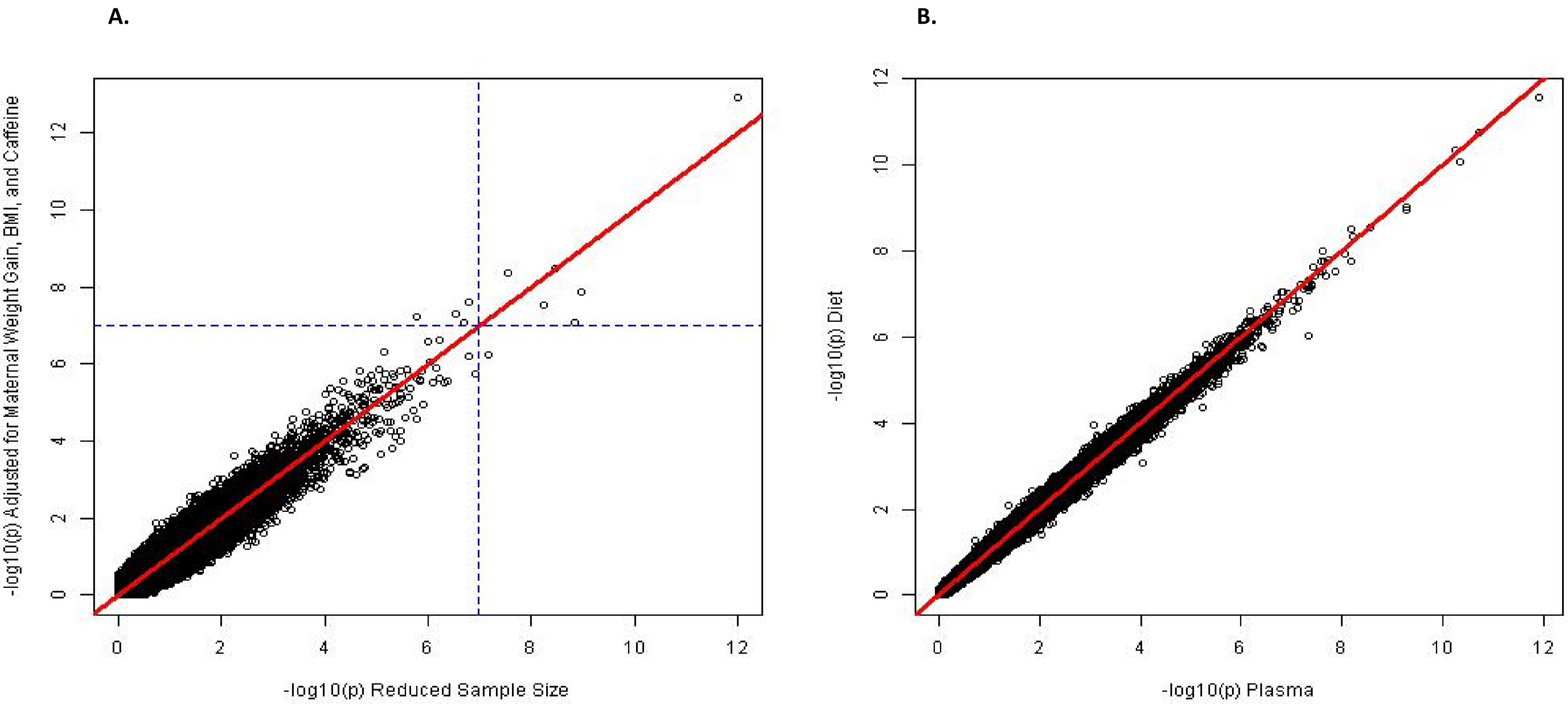
[b] Bonferroni corrected p-values = unadjusted p-value multiplied by 485,492(number of tests). The threshold for significance remains $p < 0.05$

Figure 1



−log10(p)
0
2
4
6
8
10
12
14
1
2
3
4
5
6
7
8
9
10
11
12
13
15
17
19
21
Chromosome

Figure 2 A & B



A.
B.
-log10(p) Adjusted for Maternal Weight Gain, BMI, and Caffeine
-log10(p) Reduced Sample Size
0
2
4
6
8
10
12
-log10(p) Diet
-log10(p) Plasma

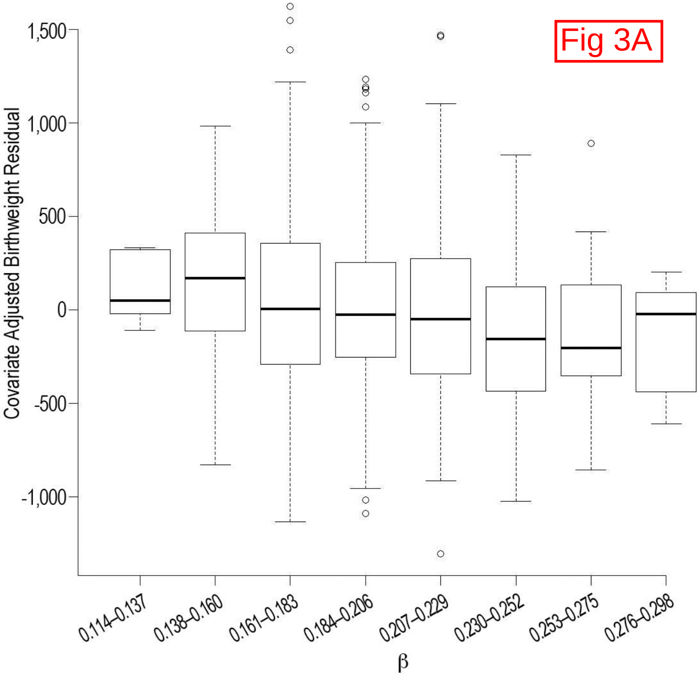


Fig 3A
Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.114–0.137
0.138–0.160
0.161–0.183
0.184–0.206
0.207–0.229
0.230–0.252
0.253–0.275
0.276–0.298
β

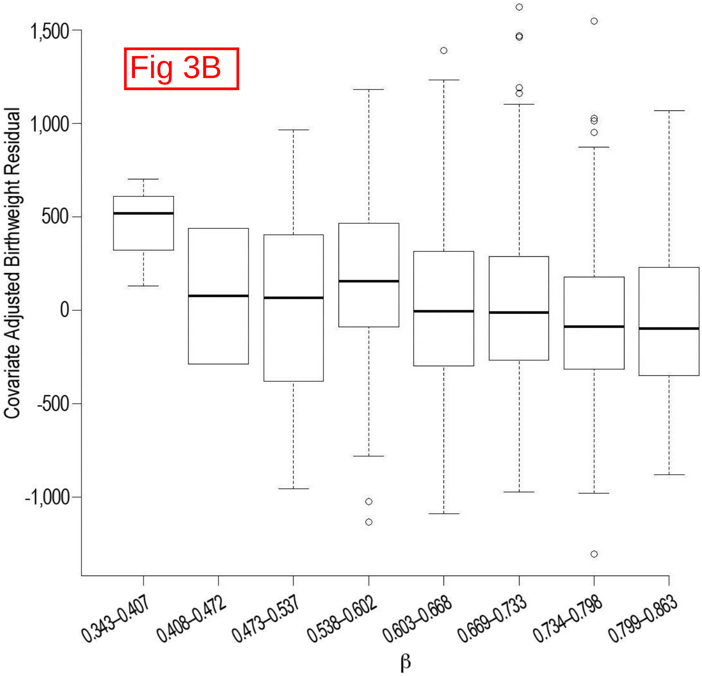


Fig 3B

Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.343–0.407
0.408–0.472
0.473–0.537
0.538–0.602
0.603–0.668
0.669–0.733
0.734–0.798
0.799–0.863
β

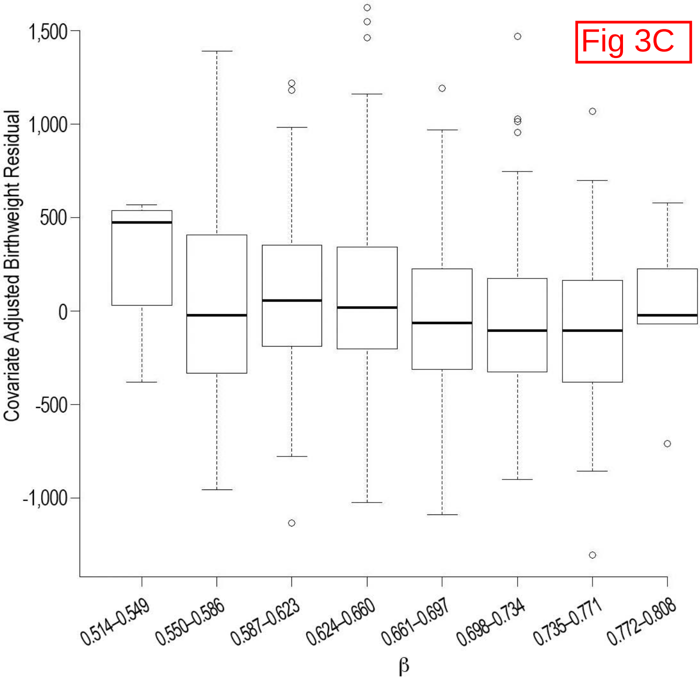

1,500
1,000
500
0
-500
-1,000
Covariate Adjusted Birthweight Residual
0.514–0.549
0.550–0.586
0.587–0.623
0.624–0.660
0.661–0.697
0.698–0.734
0.735–0.771
0.772–0.808
β

Fig 3C

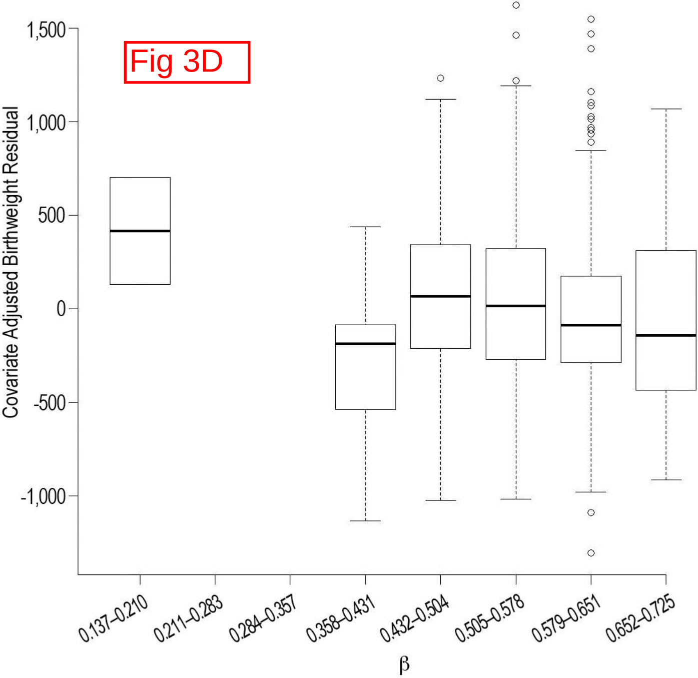
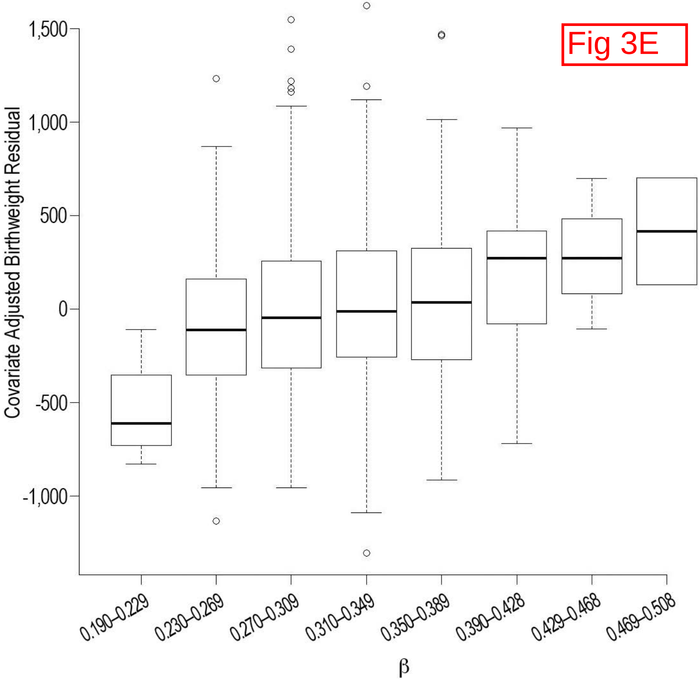


Fig 3D

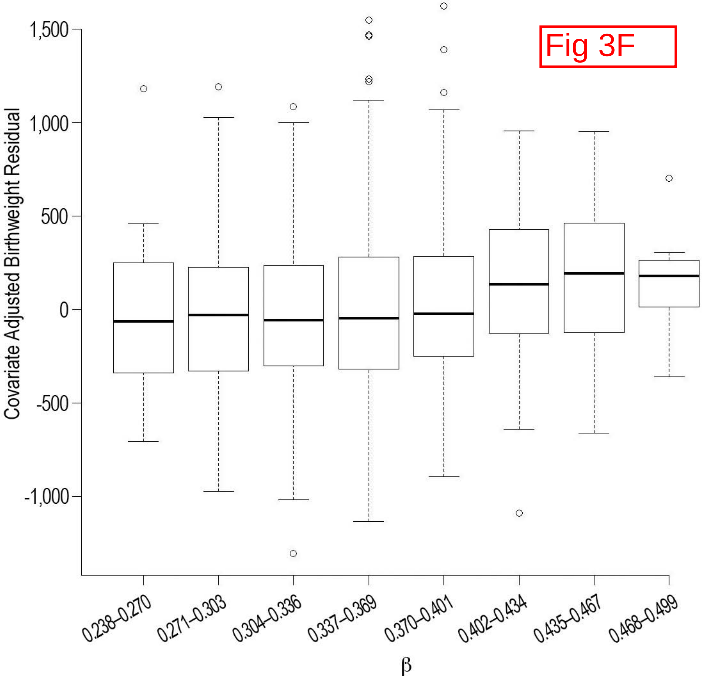
1,500
1,000
500
0
-500
-1,000
Covariate Adjusted Birthweight Residual
0.137–0.210
0.211–0.283
0.284–0.357
0.358–0.431
0.432–0.504
0.505–0.578
0.579–0.651
0.652–0.725
β

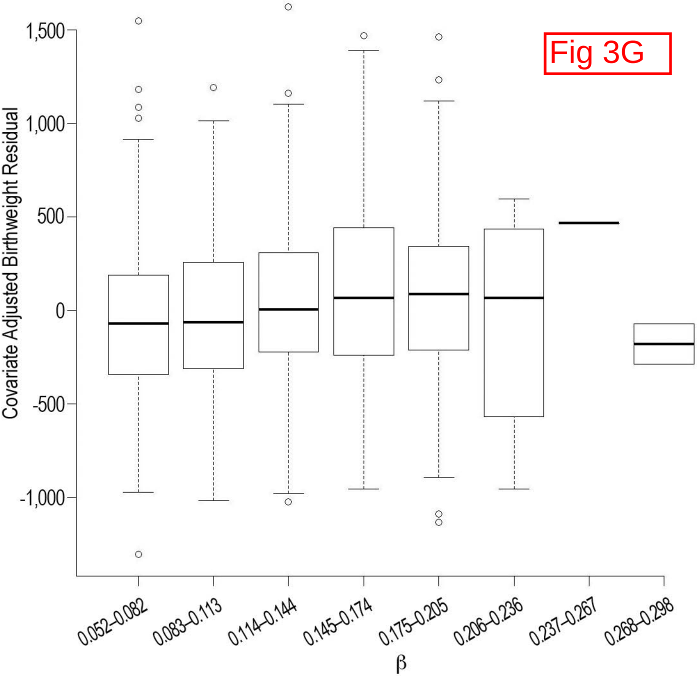
Fig 3E



Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.190–0.229
0.230–0.269
0.270–0.309
0.310–0.349
0.350–0.389
0.390–0.428
0.429–0.468
0.469–0.508
β

Fig 3F

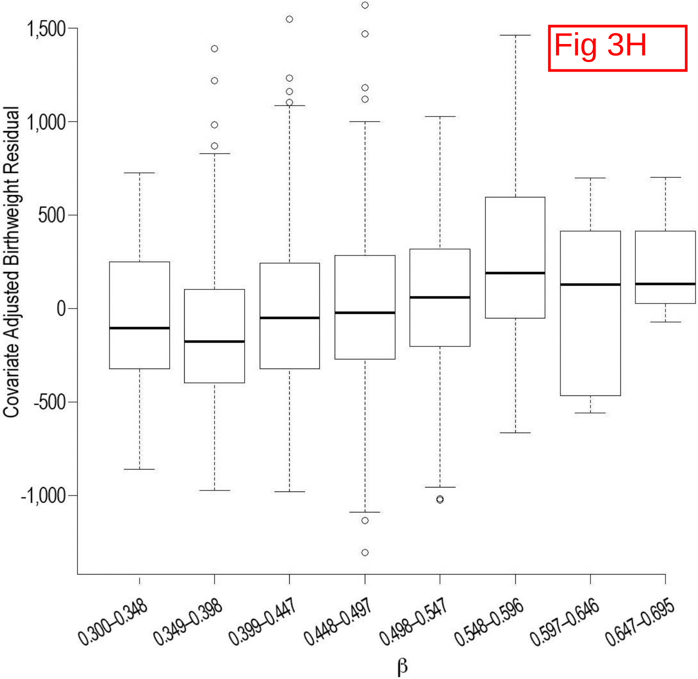

Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.238–0.270
0.271–0.303
0.304–0.336
0.337–0.369
0.370–0.401
0.402–0.434
0.435–0.467
0.468–0.499
β



Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.052–0.082
0.083–0.113
0.114–0.144
0.145–0.174
0.175–0.205
0.206–0.236
0.237–0.267
0.268–0.298
β

Fig 3G

Fig 3H


Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.300–0.348
0.349–0.398
0.399–0.447
0.448–0.497
0.498–0.547
0.548–0.596
0.597–0.646
0.647–0.695
β

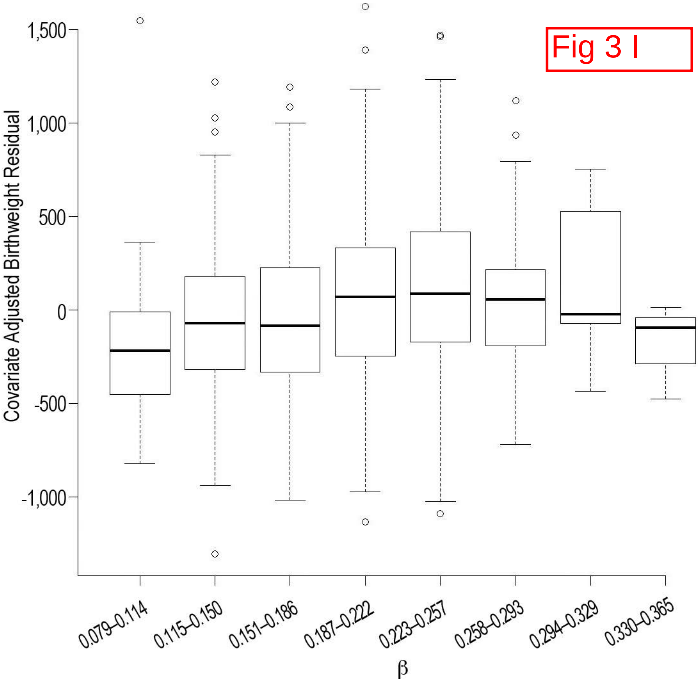

1,500
1,000
500
0
-500
-1,000
Covariate Adjusted Birthweight Residual
0.079–0.114
0.115–0.150
0.151–0.186
0.187–0.222
0.223–0.257
0.258–0.293
0.294–0.329
0.330–0.365
β

Fig 3 I

Fig 3 J

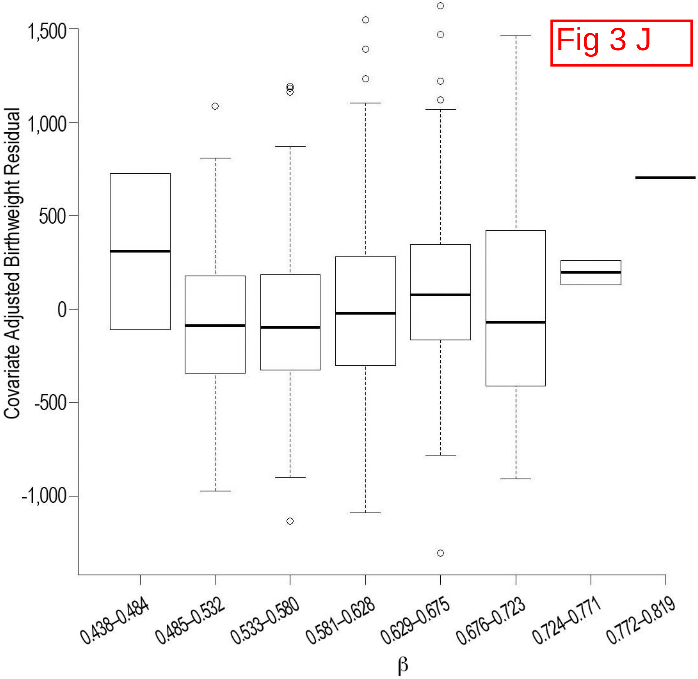

Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.438–0.484
0.485–0.532
0.533–0.580
0.581–0.628
0.629–0.675
0.676–0.723
0.724–0.771
0.772–0.819
β

Fig 3K

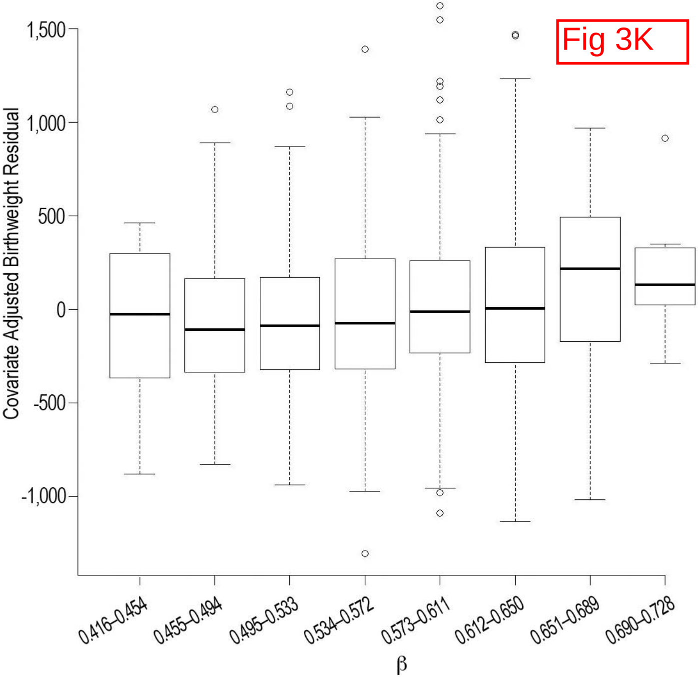
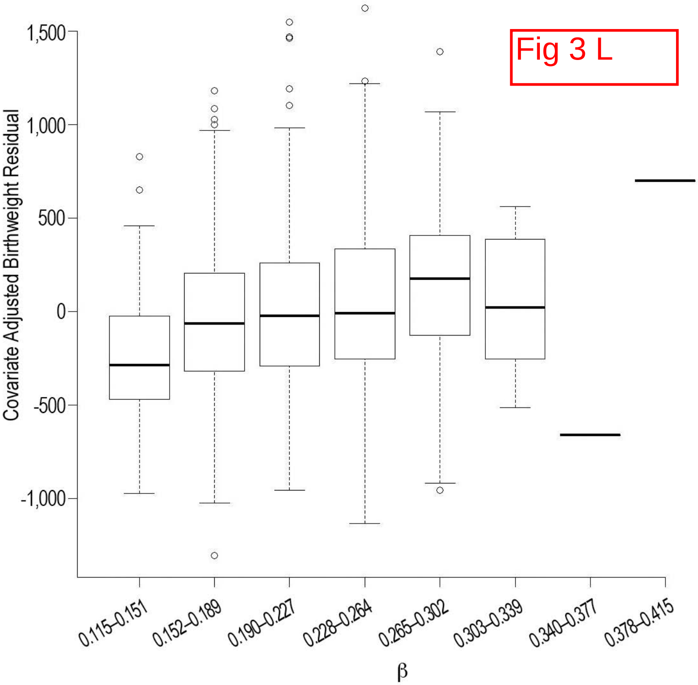

Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.416–0.454
0.455–0.494
0.495–0.533
0.534–0.572
0.573–0.611
0.612–0.650
0.651–0.689
0.690–0.728
β

Fig 3 L


Covariate Adjusted Birthweight Residual
1,500
1,000
500
0
-500
-1,000
0.115–0.151
0.152–0.189
0.190–0.227
0.228–0.264
0.265–0.302
0.303–0.339
0.340–0.377
0.378–0.415
β