Birth weight predicts psychiatric and physical health, cognitive function, and DNA methylation differences in an adult population

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Abstract

Introduction: The Developmental Origins of Adult Disease (DOAD) theory predicts that prenatal and early life events shape adult health outcomes. Birth weight is a useful indicator of the foetal experience, and has been associated with multiple adult health outcomes. DNA methylation (DNAm) is one plausible mechanism behind the relationship of birth weight to adult health.

Methods: The Generation Scotland study purposefully oversampled individuals from three historic Scottish birth cohort studies containing birthweight information, and linked additional individuals to their birth records through the NHS Information and Statistics Division. Data linkage with these sources yielded a sample of 4,710 individuals. Health and disease measures were related to birth weight in regression models. An epigenome-wide association study (EWAS) was performed in a subgroup (n=1,395), relating adult DNAm to birth weight. Replication was assessed in an independent sample (n=362)

Results: Higher birth weight was significantly associated with reduced incidence of depression, higher body mass index (BMI), lower risk of osteoarthritis, and higher general intelligence score (absolute standardised effect sizes ranged from 0.04 to 0.30, $P_{(FDR)}<0.05$). Meta-analysis of the discovery and replication EWAS studies yielded one genome-wide significant CpG site ($p=5.97x10^{-9}$), which was located in a gene linked to placental embedding.

Conclusions: Our results demonstrate associations between birth weight and adult health and functional outcomes, with particularly striking effects for depression risk. It also provides support for an association between birth weight and DNAm. This study is the first to describe an EWAS of birth weight in an adult sample.

Introduction

The Developmental Origins of Adult Disease theory (DOAD) states that through developmental plasticity, the foetal experience can permanently influence adult health [1]. The theory's main proponent, David Barker, originally relied on birth weight as an index of foetal nutrition – an assumption that has been contested by the awareness that multiple factors can influence birth weight [2]. Maternal stress, illness, and socioeconomic status [3-5] are among modifiable influences over offspring birth weight; in addition, maternal-specific genetic variation has been found to influence birth weight, acting through the intrauterine environment [6]. Thus, birth weight can be seen as an index of the general foetal experience.

There is evidence of a U-shaped association between birth weight and adult health. Birth weight is clinically conceptualised as 'low' below 2.5kg, and 'high' above 4.5kg [7], however it can also be analysed on a continuous scale. Lower birth weight has been associated with an increased risk of heart disease, type II diabetes, stroke, and hypertension [8-11]. Low birth weight is also associated with poorer cognitive ability, and a raised risk for mood disorders [12, 13]. At the higher end of the spectrum, birth weight is associated with higher body mass index, and higher risk of breast cancer [14, 15]. These associations are found after accounting for adult lifestyle factors, such as smoking and BMI, indicating a residual effect of birth weight on health outcomes. Since birth weight information is recorded as standard practice, it could be considered a useful and readily available predictive tool for adult health risks.

The DOAD theory is a plausible explanation for these observations. Prenatal factors affect the foetus in its highly plastic state, giving rise to birth weight variability, and also to developmental changes which permanently affect the function and health of organs and systems [1]. Some of these changes may be structural, affecting for example, vascular development and function [16]. DNA methylation (DNAm) is an epigenetic modification that can be influenced by genetics or by environmental factors throughout life. CpG methylation is characterised by the addition of a methyl group to cytosine nucleotides in the context of cytosine-guanine dinucleotides. DNAm changes are linked to the regulation of gene expression, providing a possible mechanism through which environmental influences may have lasting biological effects [17]. Therefore, DNAm is one putative mechanism through which developmental experience may influence adult health. Birth weight effects on the epigenome have been previously described in cord blood [18, 19] and during childhood [20], seeming to diminish into adolescence and beyond [18, 21].

Here, we describe associations between birth weight and a range of adult health conditions and risk factors, using high-quality data that improves on previous work in the field (e.g. [13]); it also reports an Epigenome-Wide Association Study (EWAS) of birthweight in an adult sample. While previous work has looked at DNAm relationships to birth weight in adults, none have performed EWAS studies in this age group, looking instead at persistence of DNAm effects from birth [18, 21]. We, therefore, hypothesise that while birth weight associated DNAm patterns may change over time, differences will still exist in adulthood.

Methods

Generation Scotland and other Cohorts

Generation Scotland (GS) is a Scottish family-based cohort n=23,690 [22]. Data were collected from participants between 2006 and 2011. 98% of GS participants gave informed consent for data linkage to routinely collected health data and to information from other Scottish population cohort studies, both current and historical. These include several with neonatal and maternity information: the Aberdeen Children of the 1950s ([23], ACONF); the Aberdeen Maternity and Neonatal Databank ([24], AMND); the Walker Birth Cohort [25]; and the Scottish Morbidity Records ([26], SMR02 – the Maternity Inpatient and Day Case record, and SMR11 – the Neonatal Inpatient dataset). Birth weight in grams, alongside gestational age at birth and twin information, was collated from these sources and linked to adult GS records for 4,713 participants (**Supplementary File 1**).

Ethics

All components of GS received ethical approval from the NHS Tayside Committee on Medical Research Ethics (REC Reference Number: 05/S1401/89). GS has also been granted Research Tissue Bank status by the Tayside Committee on Medical Research Ethics (REC Reference Number: 10/S1402/20), providing generic ethical approval for a wide range of uses within medical research.

Statistical Analyses

All analyses were conducted in R version 3.5.1 [27].

To control for the known effects of gestational age and sex on birthweight [28], we considered the residuals from a regression model in place of raw birth weight throughout:

Birth weight (g) ~ sex + gestational age

Self-reported diseases (yes/no) that were commonly reported in the cohort (prevalence >1%) included depression (prevalence 6.6%), osteoarthritis (1.6%), high blood pressure (3.4%), diabetes (1.3%), and asthma (16.6%). These were modelled against the birth weight residuals using logistic regression. Average diastolic and systolic blood pressure measurements, body mass index, high-density lipoprotein (HDL) cholesterol, and general intelligence score (derived from multiple cognitive tests - *see* ***Supplementary File 1***) were examined as continuous variables in linear regression models. Depression diagnosed using the Structured Clinical Interview for DSM-IV (SCID;[29] was also examined, as a binary yes/no for a diagnosis of major depressive disorder (**Supplementary File 1**). Three individuals who had responded 'yes' to all self-reported illnesses were excluded from analyses, leaving a final population of n=4,710. Some traits had missing values, which resulted in case-wise exclusion from the relevant regression model (**Table 1**).

Adult health and functional domains were regressed against birth weight residuals and relevant covariates in a simple model:

Trait ~ birth weight residuals + sex + age

And in a fully-adjusted model:

Trait ~ birth weight residuals + sex + age + Scottish Index of Multiple Deprivation (SIMD) quintile + lifetime smoking (ever/never) + years of education

For the general intelligence trait, years of education was removed from this fully-adjusted model as the two are highly collinear (Pearson r=0.36).

Correction for multiple testing was carried out using a false discovery rate P<0.05.

Epigenome-wide association study

Genome-wide DNA methylation was profiled in 5,190 individuals in GS, taken from peripheral whole blood, using the Illumina HumanMethylationEPIC BeadChip (Illumina Inc., San Diego, CA). Quality control and normalisation were carried out as described elsewhere ([30, 31]; **Supplementary File 2**). For the subgroup of GS for whom birth weight and gestational age information were available, DNAm data was available for n=1,395.

In a second release of data generated during this analysis, using a near identical protocol (**Supplementary File 2**), an independent set of methylation data became available for an additional 4,450 GS participants. In this replication sample, a further 362 participants with both birth weight and gestational age information were used.

The birth weight residuals described above were used in the EWAS model, which was run using the 'limma' package in R (empirical Bayes moderated t-statistics). The discovery EWAS model used CpGs corrected for relatedness (**Supplementary File 2**), as the first batch of DNAm data was collected on related individuals:

> CpG ~ birth weight residuals + age + sex + ever smoked (Yes/No) + Pack years smoking + 20 methylation PCs

Additional covariates (estimated white blood cell proportions – CD4T, CD8T, Granulocytes, BCells, Natural Killer cells – and methylation batch), which were regressed out during the relatedness pre-correction for the discovery dataset, were also included in the replication dataset of unrelated individuals.

Meta-Analysis of discovery and replication samples

A standard error-weighted meta-analysis of the discovery and replication EWASs was performed using the METAL software package. Summary statistics from the discovery, replication and meta-analysis EWASs are available at [**weblink to be inserted upon acceptance**].

Results

Phenotype population characteristics

There were 4,710 GS participants with birth weight and gestational age information available (**Table 1**). The population was 57% female, with a mean birth weight of 3.40kg (SD=0.52), and gestational age of 39.8 weeks (SD=1.7). The minimum birth weight in the sample was 0.7kg, the maximum was 5.1kg. Using the clinical cut-off of 2.5kg, 3.99% (n=188) of the sample were of clinically 'low' birth weight. This means the data describe phenotypic relationships to birth weight across pathological and non-pathological cases.

Relationship of adult outcomes to birth weight

Regression models identified significant associations between higher birth weight (effect sizes are reported per SD) and lower risk of SCID-diagnosed depression (OR=0.86; 95% CI 0.78-0.95, p=0.018);

lower risk of self-reported osteoarthritis (OR=0.74; 95% CI 0.58-0.94; p=0.034); higher BMI (β=0.072; SE=0.016, $p=3.7x10^{-05}$) and higher general intelligence (β=0.042; SE=0.016, p=0.027; **Table 2**).

In minimally-adjusted regression models (covarying for age and sex alone), higher birth weight showed a relationship with lower self-reported depression risk (OR=0.85; 95% CI 0.75-0.96; p=0.018). Associations were also found for the four traits described above, but no other adult health or functional outcomes (**Supplementary File 3**).

Epigenome-wide association study of birthweight

The epigenome-wide association study of birth weight revealed no CpGs significant at the genome-wide level ($P<3.6x10^{-8}$; [32]), although 19 CpGs had $P<1x10^{-5}$(minimum FDR corrected P-value of $6.05x10^{-8}$ for cg00966482) (**Figure 1A; Supplementary File 4**). The 19 CpGs were largely uncorrelated, with the exception of three CpGs (located within *CASZ1* – two within 200 base pairs of each other, with the third site around 11kb away – **Supplementary File 4**) that had absolute r≥0.6(**Figure 2**).

Replication in an independent DNAm sample

There were no genome-wide significant associations in the replication sample (n=362) (**Figure 1B; Supplementary File 5**).

Of the 19 CpGs that exceeded the suggestive significance threshold in the discovery EWAS, two reached nominal significance (P<0.05) in the replication analysis: cg00590817 (p=0.0069), and cg00966482 (p=0.031). The latter CpG was the most significantly associated site from the discovery EWAS ($p=6.05x10^{-8}$) and is found within the *HERV-FRD* (also referred to as *ERVFRD-1*) gene. There was good concordance between the effect sizes of DNAm associations with birthweight in the discovery and replication studies (r=0.59) (**Figure 3**).

Meta-analysis of discovery and replication samples

Meta-analysis of the original discovery EWAS sample with the replication sample found a genome-wide significant effect of birthweight on DNAm at the CpG site cg00966482 located in the *ERVFRD-1* gene ($p=5.97x10^{-9}$; **Figure 1C**; **Supplementary File 6**).

Discussion

We observed a strong association between birth weight and adult depression, where a 1 standard deviation (SD; 0.52kg) increase in birth weight was linked to 15% lower odds of future depression. A 1SD increase in birth weight was also linked to a 0.072 SD (~0.37kg/m^2) higher adult BMI, a 0.042 SD higher general intelligence score, and 30.7% lower odds for self-reported osteoarthritis. We also identified one genome-wide significant association between birth weight and blood-based DNA methylation in adulthood.

Birth weight and depression

Previous studies have found both positive and negative associations between birth weight and depression. A meta-analysis of 18 studies (n=~50,000) found support for a modest effect of low birthweight (<2.5kg) on depression risk (OR=1.15, 95% CI=1.00-1.32), but suggested this was a result of publication bias [13]. We improved upon the design of many of the papers within that meta-analysis by utilising clinician-diagnosed depression and birth weight as a continuous variable (compared to self-reported measures of depression or psychological distress and use of a binary (<2.5kg vs normal) birth weight variable). Compared to SCID-diagnosed depression, we found self-reported depression had a weaker association with birth weight. Furthermore, the association with the self-reported measure was statistically significant in the minimally-adjusted but not fully-adjusted regression models. This emphasises the importance of using high quality, clinically meaningful tools for measuring psychiatric disorders. The aetiology of depression is complex, with multiple biological and psychosocial factors contributing increments of risk, thought to sum towards illness. This study indicates that birth weight may be considered as one of these contributing risk factors, as its association with adult diagnosis remained after accounting for many adult lifestyle factors.

Birth weight and other outcomes

Consistent with the published literature [11, 14], we identified an association between higher birth weight and higher adult BMI. Previous work has used Mendelian randomisation techniques to show that birthweight is a causal factor in determining adult BMI [9]. We also found a significant relationship between higher birth weight and lower prevalence of osteoarthritis in this sample. Developmental origins of osteoarthritis have been described before, with higher disease prevalence in low birth weight individuals [33], and those with low weight at age one [34].

A positive association between birth weight and general intelligence is also reported here (1SD increase in birthweight results in a 0.042 SD higher general intelligence score). Impaired cognitive

ability after low birth weight has been shown in several samples in childhood [12] and into adulthood [35].

Some previously-established effects of birth weight on adult health are not supported here, for instance, links to type 2 diabetes and cardiovascular disease [9]. This may be partly due to the self-report nature of the health measures used. Our sample size was also smaller than previous investigations into birth weight and cardiovascular outcomes [9, 11].

Epigenome-wide association study of birth weight in adult samples

In the EWAS meta-analysis we observed a genome-wide significant association between higher birthweight and higher methylation levels at cg00966482 (*ERVFRD-1*). *ERVFRD-1* encodes syncitin-2, a protein involved in placental embedding [36]. This site was not identified in a previous meta-analysis EWAS of birthweight [21]. Moreover, this is the first report of a genome-wide significant EWAS association for birth weight in an adult sample.

Of the 19 CpG sites with $P<1x10^{-5}$ in the discovery EWAS, 10 were located within known genes. Some of these genes contain SNPs that have genome-wide associations (with $P<5x10^{-8}$) with cardiovascular, psychiatric, and developmental pathways (**Supplementary File 7**). Three of the 19 nominally significant CpG sites identified in the discovery EWAS were highly correlated (min r=0.69). Higher birthweight was associated with higher methylation levels at these sites, which were located within *CASZ1*, a gene encoding the zinc finger protein castor homolog 1, a transcriptional activator involved in vascular morphogenesis [37]. *CASZ1* was recently identified as differentially methylated in placental tissue between infants born small vs. large for gestational age [19], thus this study suggests the persistence of differential methylation of this gene into adulthood. Genetic variants in *CASZ1* have previously been implicated in GWAS studies on various aspects of cardiovascular health (**Supplementary File 7**). These have included studies in multi-ethnic populations on blood pressure [38, 39], and on other cardiovascular health issues such as atrial fibrillation [40] and stroke [41].

A recent longitudinal meta-analysis [21] of DNAm relationships to birth weight identified 914 CpG sites associated with birth weight in 24 EWAS studies from neonatal blood (total n=8,825). The persistence of methylation differences at these sites was then examined in other cohort data from childhood (total n=2,756 from 10 studies; 2-12y), adolescence (total n=2,906 from 6 studies; 16-18y), and adulthood (1,616 from 3 studies; 30-45y). Nominally significant methylation differences were found to persist into childhood, adolescence, and adulthood at 87, 49, and 42 sites respectively. This

supports evidence that birth weight-related methylation differences may attenuate over time [18, 20]. It has, however, been demonstrated that some methylation patterns persist into late adulthood after prenatal famine exposure [42]. It is therefore plausible that other prenatal factors may continue to affect DNAm into adulthood.

Strengths and Limitations

This study exploited rarely available data linkage capacity to acquire neonatal information from birth medical records. The phenotyping and DNAm data available within the cohort have allowed the association of both health traits and DNAm data with birth weight. There are, however, some limitations inherent to the cross-sectional design of this study. Longitudinal data would allow analysis of the persistence of DNAm signatures across time. In addition, the number of individuals for whom accurate birth weight and gestational information could be identified in historical health cohorts limited the sample size for the EWAS analyses.

Conclusions

This study presents the first epigenome wide association study of birth weight on DNA methylation in adulthood. We also present a comprehensive study of birth weight in relation to cognitive, psychiatric, and disease traits in mid-life. The Developmental Origins of Adult Disease theory predicts that birth weight can affect many domains of adult physical and psychological health. We found evidence to support this at both the molecular and broad disease/health phenotype levels.

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Table 1: Population characteristics of the phenotyped and EWAS samples.

	Phenotype Sample			EWAS Sample		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Age (years)	4,710	29.5	10.9	1,395	37.1	14.7
Birthweight (g)	4,710	3399	516	1,395	3377	518
Gestation (weeks)	4,710	39.8	1.7	1,395	40	1.8
BMI (kg/m2)	4,385	25.2	5.2	1,387	26.1	5.4
Education*	4,411	5	4-6	1,317	5	3-6
	N	**%**		**N**	**%**	
Sex - Male	2,025	43.0		569	40.8	
Female	2,688	57.0		826	59.2	
Socieconomic Status**	*(4,389)*			*(1,310)*		
Quintile 1 (most deprived)	621	14.2		207	15.8	
Quintile 2	717	16.3		210	16.0	
Quintile 3	715	16.3		183	14.0	
Quintile 4	1064	24.2		301	23.0	
Quintile 5 (least deprived)	1272	29.0		409	31.2	
Smoking	*(4,525)*			*(1,344)*		
Current Smoker	918	20.3		257	19.1	
Ex-Smoker (<12 months)	237	5.2		56	4.2	
Ex-Smoker (>12 months)	662	14.6		273	20.3	
Never Smoker	2,708	59.8		758	56.4	

* Median and Interquartile range reported. Education was coded as an ordinal variable: 0 = 0yrs, 1 = 1-4yrs, 2 = 5-9yrs, 3 = 10-11yrs, 4 = 12-13yrs, 5 = 14-15yrs, 6 = 16-17yrs, 7 = 18-19yrs, 8 = 20-21yrs, 9 = 22-23yrs, 10 = ≥24yrs.

**SIMD Quintile. SIMD is the Scottish Index of Multiple Deprivation, a postcode-derived index of socioeconomic status. The quintiles derived on the full Generation Scotland cohort ranged from 1 (most deprived) to 5 (least deprived).

Table 2: Outputs of logistic and linear regression models of health traits ~ birth weight residuals + age + sex + socioeconomic status (SIMD) + ever smoke + education years, with FDR-correction for multiple testing.
*For the 'g' general intelligence factor, education years was removed from the model as it resulted in over-correction of the effect.

Trait	**Odds Ratio**	**95% CI**	**P value**	**P (FDR)**
SCID depression	0.86	0.78 - 0.95	0.0033	0.018
SR depression	0.92	0.81 - 1.05	0.21	0.38
SR hypertension	0.93	0.78 - 1.09	0.36	0.44
SR diabetes	0.96	0.74 - 1.25	0.76	0.76
SR osteoarthritis	0.74	0.58 - 0.94	0.012	0.034
SR asthma	0.96	0.88 - 1.04	0.3	0.43
Trait	**Beta**	**SE**	**P value**	**P (FDR)**
Body Mass Index (kg/m^2)	0.072	0.016	$3.4x10^{-6}$	$3.7x10^{-5}$
HDL cholesterol	0.025	0.016	0.12	0.27
Average systolic BP	-0.014	0.014	0.32	0.43
Average diastolic BP	-0.011	0.015	0.47	0.51
General intelligence*	0.042	0.016	0.0074	0.027

Figure 1: Manhattan plots for the epigenome-wide association study of birth weight (**A**); the replication sample EWAS (**B**); and the meta-analysis of the main EWAS and the replication (**C**). The black and red lines represent the suggestive, and Bonferroni corrected P-value thresholds of $P=1x10^{-5}$ and $3.6x10^{-8}$, respectively.

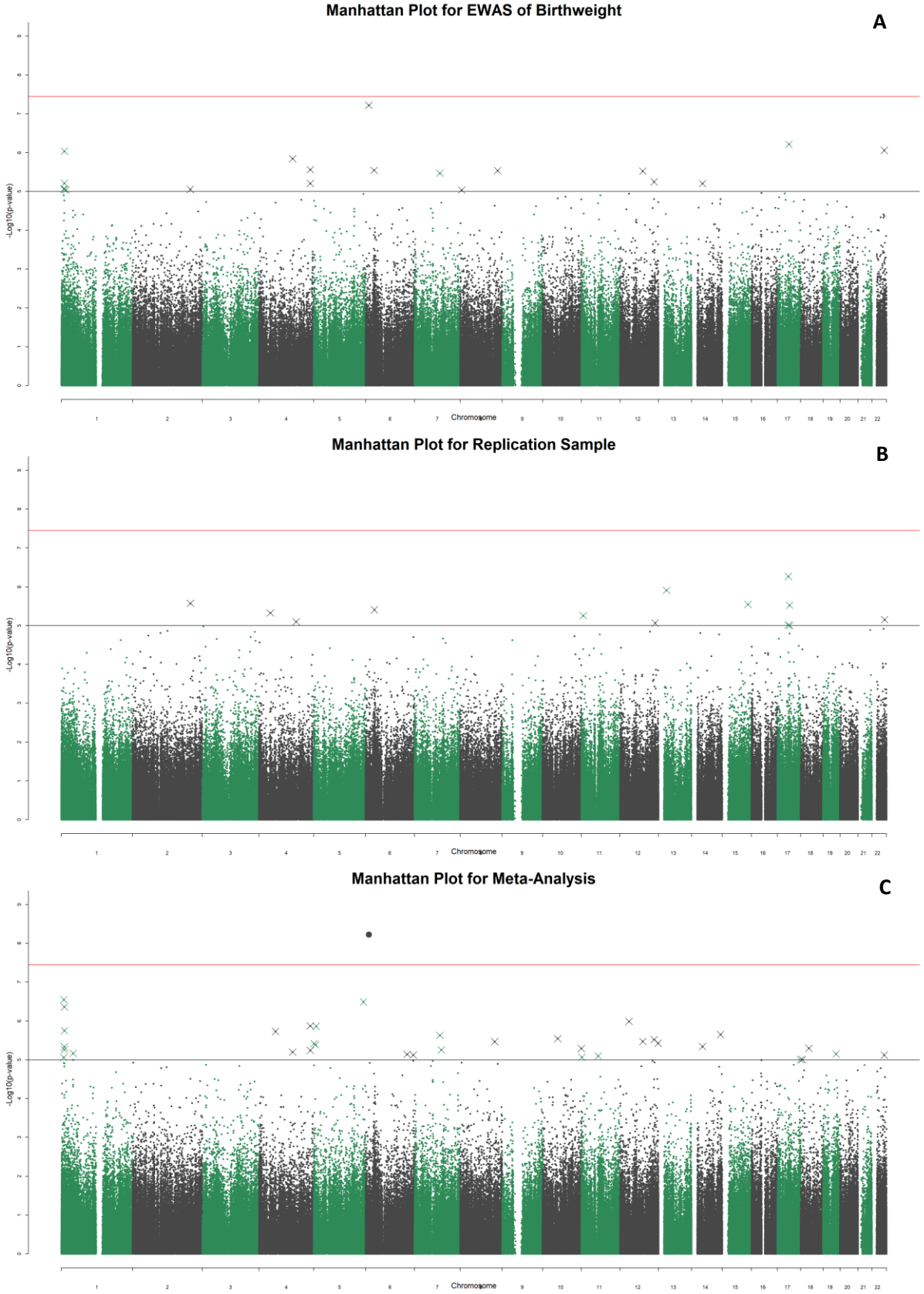

Figure 2: Correlation plot between methylation at the top 19 CpG sites from the epigenome-wide association study. The shade and scale of the dots represent the magnitude and direction of the correlation between pairs of CpGs.

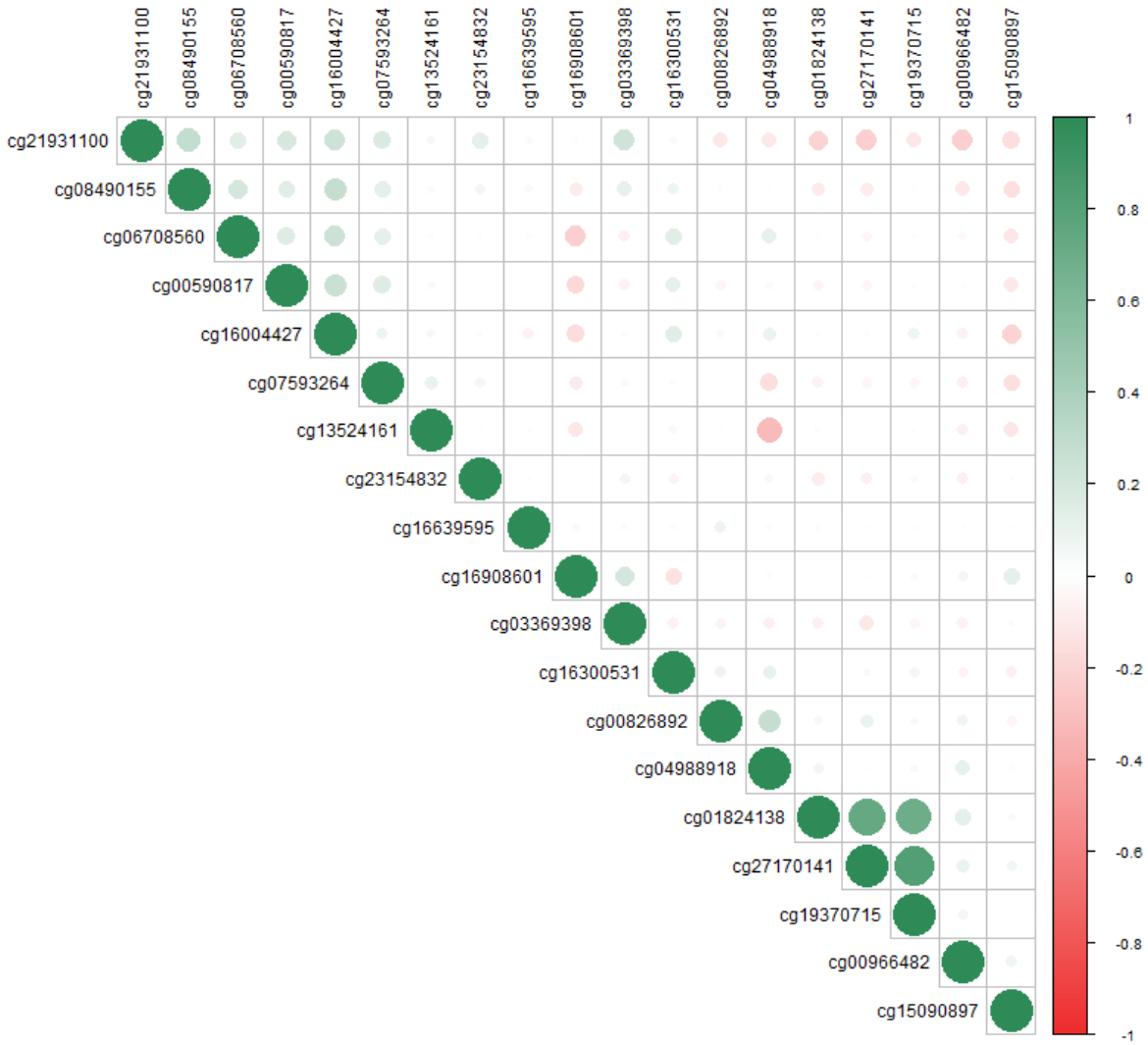

Figure 3: Effect sizes for 18 of the top 19 CpG sites in the discovery sample plotted against the effect sizes in the replication sample (cg04988918 was not included in the replication array). The point size is determined by the -log10 of the p-values for these hits in the replication analysis. The two points labelled in black are the two CpG sites which achieved nominal significance in the replication study, and the three highlighted in red are the three co-methylated CpG sites within the *CASZ1* gene.