**Pre-publication data release policy – the GoDMC consortium**

**Project Proposal**

*Submitted to the GoDMC consortium:* 2021-04-13

*Project Title:* MR analysis to examine causal associations of cord blood vitamin B12 concentrations on cord blood DNA methylation

*Aim:* Perform a two-sample Mendelian Randomization analysis to assess causality in the association of cord blood B12 concentrations with cord blood DNA methylation.

Abstract:

Within the Pregnancy And Childhood Epigenetics (PACE) Consortium we have conducted a meta-analysis of epigenome-wide association studies investigating associations of circulating cord blood vitamin B12 concentrations and cord blood DNA methylation. This meta-analysis resulted in 7 FDR-significant CpGs in relation to cord blood vitamin B12 concentrations. To examine whether these associations reflect a causal effect of cord blood vitamin B12 concentrations on cord blood DNA methylation, we aim to use a two-sample Mendelian randomization (MR) meta-analysis approach. We selected 10 SNPs based on their robust associations at genome-wide significance with circulating vitamin B12 concentrations, as reported by a genome-wide association study (GWAS) among 45,576 individuals from Denmark and Iceland (1). Using multiple independent SNPs likely increases power, as they collectively will explain more of the variance in vitamin B12 concentrations (2). We aim to use these SNPs as genetic instruments for vitamin B12 levels and to examine their associations with DNA methylation at the 7 identified CpGs.

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**Data requested from the GoDMC Study**

***Only summary statistics can be requested***

We request summary statistics of all 7 CpGs and 10 SNPs (or proxies) listed in **Tables 1** and **2**.

*Analysis plan*

**Instruments**

The 10 SNPs (**Table 2**) that we selected based on their robust associations at genome-wide significance with circulating vitamin concentrations, as reported by a genome-wide association study (GWAS) among 45,576 individuals from Denmark and Iceland (2). An additional SNP reported in this GWAS was monomorphic in Europeans and therefore excluded from the analyses.

**Outcomes**

Cord blood DNA methylation (beta values) from Illumina Infinium HumanMethylation450 at the 7 CpGs that were differently methylated in relation to cord blood vitamin B12 concentrations in the PACE meta-analysis.

**Summary statistics**

We will use summary statistics from the GoDMC database. For SNPs not available in GoDMC, we will include proxies (R2>0.8).

**Analysis**

As done in previous literature using the same SNPs as instruments, we will conduct MR analyses with and without the known pleiotropic variant rs602662 (*FUT2*) (2). Analyses will be conducted in the one-sample MR package in R.

*Time line:*

Project start: April 2021

Project end: June 2021

*Proposed authors (Please see authorship policy):*

We suggest to add 2-3 GoDMC authors as named authors on the paper and to acknowledge GoDMC’s contribution in supporting and encouraging the collaboration in the acknowledgements section of the manuscript.

*Title and summary for GoDMC website:*

Title: Mendelian randomisation analysis to examine causal associations of cord blood vitamin B12 concentrations on cord blood DNA methylation

Summary:

Within the Pregnancy And Childhood Epigenetics (PACE) Consortium we have conducted a meta-analysis of epigenome-wide association studies investigating associations of circulating cord blood vitamin B12 concentrations and cord blood DNA methylation. This meta-analysis resulted in 7 FDR-significant CpGs in relation to cord blood vitamin B12 concentrations. To examine whether these associations reflect a causal effect of cord blood vitamin B12 concentrations on cord blood DNA methylation, we aim to use a two-sample Mendelian randomization (MR) meta-analysis approach. We selected 10 SNPs based on their robust associations at genome-wide significance with circulating vitamin B12 concentrations, as reported by a genome-wide association study (GWAS) among 45,576 individuals from Denmark and Iceland (1). Using multiple independent SNPs likely increases power, as they collectively will explain more of the variance in vitamin B12 concentrations (2). We aim to use these SNPs as genetic instruments for vitamin B12 levels and to examine their associations with DNA methylation at the 7 identified CpGs.

**REFERENCES**

1. Grarup N, Sulem P, Sandholt CH, Thorleifsson G, Ahluwalia TS, Steinthorsdottir V, et al. Genetic Architecture of Vitamin B12 and Folate Levels Uncovered Applying Deeply Sequenced Large Datasets. PLOS Genetics. 2013;9(6).
2. Moen G-H, Qvigstad E, Birkeland KI, Evans DM, Sommer C. Are serum concentrations of vitamin B-12 causally related to cardiometabolic risk factors and disease? A Mendelian randomization study. The American Journal of Clinical Nutrition. 2018;108(2):398-404.

**Table 1: 7 CpGs that were significantly associated with neonatal vitamin B12 concentrations in cord blood (FDR <0.05 and I2 <50%).**

|  |
| --- |
| cg13863764 |
| cg00658405 |
| cg08243619 |
| cg24371425 |
| cg02615136 |
| cg04096723 |
| cg09573658 |

**Table 2: 10 SNPs that have been associated with vitamin B12 concentrations1.**

|  |  |  |  |
| --- | --- | --- | --- |
| SNP | Effect allele2 | Other allele | Potential proxies3 (R2 >0.8) |
| rs2336573 (*CD320*) | T | C | N=59 (Table S1) |
| rs1131603 (*TCN2*) | C | T | N=1 (Table S1) |
| rs3742801 (*ABCD4*) | T | C | N=39 (Table S1) |
| rs2270655 (*MMAA*) | G | C | N=48 (Table S1) |
| rs12272669 (*MMACHC*) | A | G | N=94 (Table S1) |
| rs34324219 (*TCN1*) | C | A | Not available |
| rs602662 (*FUT2*) | A | G | N=37 (Table S1) |
| rs1801222 (*CUBN*) | G | A | Not available |
| rs41281112 (*CLYBL*) | C | T | Not available |
| rs1141321 (*MUT*) | C | T | N=90 (Table S1) |

1. rs7788053 (*FUT6*) was excluded from analyses as this SNP is mono-allelic in European populations.
2. The allele associated with increased serum vitamin B-12 concentrations
3. Proxies can be obtained from: <https://ldlink.nci.nih.gov/?tab=ldproxy> (Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015 Jul 2.)