

**BRIEF COMMUNICATION**

**Open Access**

# Vitamin B<sub>12</sub> deficiency in the brain leads to DNA hypomethylation in the TCblR/CD320 knockout mouse

Sílvia Fernàndez-Roig<sup>1,2</sup>, Shao-Chiang Lai<sup>3</sup>, Michelle M Murphy<sup>1,2</sup>, Joan Fernandez-Ballart<sup>1,2</sup> and Edward V Quadros<sup>3,4\*</sup>

## Abstract

**Background:** DNA methylation is an epigenetic phenomenon that can modulate gene function by up or downregulation of gene expression. Vitamin B<sub>12</sub> and folate pathways are involved in the production of S-Adenosylmethionine, the universal methyl donor.

**Findings:** Brain vitamin B<sub>12</sub> concentration and global DNA methylation was determined in transcobalamin receptor (TCblR/CD320) knock out (KO) (n = 4) and control mice (n = 4) at 20–24 weeks of age. Median [IQR] brain vitamin B<sub>12</sub> concentrations (pg/mg) in TCblR/CD320 KO mice compared with control mice was 8.59 [0.52] vs 112.42 [33.12]; p < 0.05. Global DNA methylation levels in brain genomic DNA were lower in TCblR/CD320 KO compared with control mice (Median [IQR]: 0.31[0.16] % vs 0.55[0.15] %; p < 0.05).

**Conclusions:** In TCblR/CD320 KO mice, brain vitamin B<sub>12</sub> drops precipitously by as much as 90% during a 20 week period. This decrease is associated with a 40% decrease in global DNA methylation in the brain. Future research will reveal whether the disruption in gene expression profiles due to changes in DNA hypomethylation contribute to central nervous system pathologies that are frequently seen in vitamin B<sub>12</sub> deficiency.

**Keywords:** Vitamin B<sub>12</sub> deficiency, Brain, Global DNA methylation, Transcobalamin receptor knock out mice

## Findings

### Background

Vitamin B<sub>12</sub> and folate participate in a number of biochemical reactions that affect methyl group production and transfer. Vitamin B<sub>12</sub> is a cofactor for methionine synthase (MS) that catalyses the conversion of homocysteine to methionine. Methionine is then converted to S-adenosylmethionine (SAM), a universal methyl group donor [1] for methylation of DNA and RNA. The transfer of a methyl group to the 5-carbon cytosine residue by SAM is catalysed by DNA methyltransferase enzymes [2].

DNA methylation is a fundamental mechanism for the epigenetic control of gene expression and the maintenance

of genomic integrity [3]. DNA hypermethylation can lead to transcriptional silencing by blocking transcription factors [4] and DNA hypomethylation to genomic instability and the expression of genes that would be otherwise suppressed [5]. DNA methylation plays a critical role in the generation of oncogenic point mutations, silencing of tumor suppressor genes and in the activation of the cancer process [6].

Low vitamin B<sub>12</sub> status results in reduced MS activity, impaired remethylation of homocysteine to methionine and subsequent SAM production. Consequently, methylation reactions are impaired and DNA methylation capacity is reduced [7]. Severe vitamin B<sub>12</sub> deficiency leads to neurological manifestations such as myelopathy, neuropathy, dementia and neuropsychiatric abnormalities [8] and to demyelination in the central nervous system [9]. DNA hypomethylation could be a link between vitamin B<sub>12</sub> deficiency and neurological pathology. Although the association was not maintained in multivariate

\* Correspondence: edward.quadros@downstate.edu

<sup>3</sup>Departments of Medicine and Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY, USA

<sup>4</sup>Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA

Full list of author information is available at the end of the article

analysis, preliminary analysis showed that low tissue B<sub>12</sub> concentrations were associated with DNA hypomethylation in human squamous cell lung cancer [10]. B<sub>12</sub> deficient diet has decreased DNA methylation and increased uracil incorporation in rats [11]. DNA hypomethylation has been reported in fetal brains with neural tube defects [12]. The transcobalamin receptor (TCbIR)/*CD320* knock out (KO) mouse provides a model to investigate the relationship between severe B<sub>12</sub> deficiency and DNA methylation status.

### Hypothesis

Considering the role of vitamin B<sub>12</sub> in recycling of folate and *de novo* methionine production, our hypothesis is that vitamin B<sub>12</sub> deficiency in the brain causes global DNA hypomethylation in the TCbIR/*CD320* mouse.

### Methods

#### Animals

The TCbIR KO mouse ES cells (+/-) generated by gene trap technology at the Sanger Institute were obtained from the Mouse Genome Center, UC Davis, CA. The *CD320* gene KO was produced in the C57Bl/6 N strain using heterozygous ES cells. The KO mice were produced in the laboratory of R Finnell, Texas Institute of Genomic Medicine, Houston, TX. The null TCbIR/*CD320* KO mouse was generated by cross breeding. The production of *CD320* KO mice and breeding was done as per NIH guidelines and the protocol was approved by the AALAC accredited institutional animal care and use committee. The mice were bred and fed on standard chow at SUNY Downstate Medical Center (Brooklyn, New York) for the study. The initial genotyping was done by PCR using genomic DNA extracted from mouse tail. In this study, four control and four TCbIR/*CD320* null mice were analyzed. The animals were euthanized by CO<sub>2</sub> inhalation before being sacrificed at age 20–24 weeks. The whole brain was removed and quick-frozen in liquid nitrogen. After dissection, the brain tissue was stored at -80°C.

#### Preparation of mouse tissue samples for total vitamin B<sub>12</sub> assay

Brain tissue was homogenized in two volumes of distilled water based on wet weight of tissue. 100 µl of the homogenized tissue was mixed with nine volumes of vitamin B<sub>12</sub> extraction buffer (one volume of 0.06 M citrate-phosphate buffer, pH 2.6, one volume of 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4 and one volume of KCN, 100 µg/ml; final pH of extraction buffer is 4.5); in a tightly capped glass tube and placed in a boiling water bath for 30 minutes to extract vitamin B<sub>12</sub>. The sample was briefly centrifuged after being cooled to room temperature and transferred to a 1.5 ml microcentrifuge tube. The precipitated proteins were pelleted by spinning at 14,000 rpm for 15 minutes and the clear extract

was collected and neutralized by mixing one-third of a volume of 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, pH 9.0. Total vitamin B<sub>12</sub> was assayed as previously described [13].

#### Determination of global DNA methylation in the brain

Genomic DNA from 25 mg of brain tissue was purified with the *DNeasy blood & tissue kit* (Qiagen, Inc). DNA concentration and purity was determined by comparing the ratio of optical density measurements at 260 and 280 nm. Global DNA methylation was determined using the Methyl Flash Methylated DNA Quantification Kit (Colorimetric) (Epigentek Group Inc., New York, NY, USA) [14]. The kit measures the methyl-cytosine content as a percentage of total cytosine content. Using a DNA concentration of 20 µg/mL, the purified DNA was added to ELISA plate. The methylated fraction of DNA is quantified using 5-methylcytosine specific antibodies. The amount of methylated DNA was proportional to the OD intensity in an ELISA plate reader at 450 nm. DNA methylation was calculated using the formula: [(sample OD-M3OD)/S]/[((M4OD-M3OD)x2)/P]x100; where **OD** is optical density; **M3** is the negative control, an unmethylated polynucleotide containing 50% of cytosine; **S** is the amount of input sample DNA in ng; **M4** is the positive control, a methylated polynucleotide containing 50% of 5-methylcytosine; **P** is the amount of input positive control in ng. The amount of methylated DNA was expressed as percentage of total DNA.

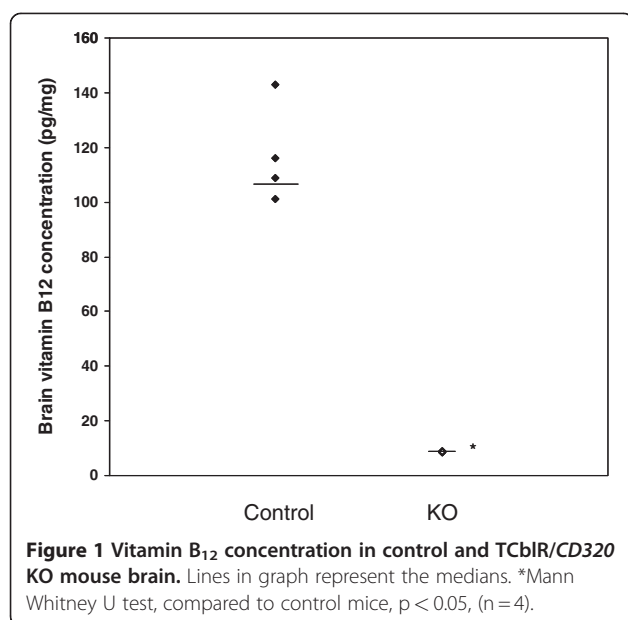
#### Statistical analysis

Vitamin B<sub>12</sub> concentration and global DNA methylation are reported as median [IQR]. Both determinations were compared between control and TCbIR/*CD320* KO mice groups with the non parametric one-tailed Mann Whitney U test. A p-value <0.05 was considered significant. The data were analyzed using SPSS (*Statistical Package for Social Sciences, Chicago, IL, USA*) version 17.0.

#### Results and discussion

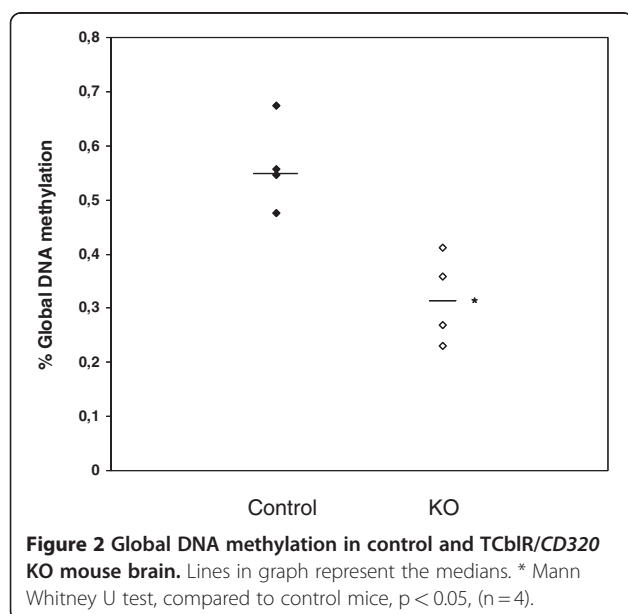
Median [IQR] brain vitamin B<sub>12</sub> concentrations in TCbIR/*CD320* KO mice were lower than in control mice: 8.6 [0.52] pg/mg vs 112.4 [33.12] pg/mg; p < 0.05 (Figure 1). Brain vitamin B<sub>12</sub> concentration was 92% lower in TCbIR/*CD320* KO mouse brains than in controls. Median [IQR] global genomic DNA methylation levels were significantly lower in TCbIR/*CD320* KO (0.31 [0.16] %) than control mice brains (0.55 [0.15] %) (p < 0.05) (Figure 2). Global DNA methylation was 44% lower in TCbIR/*CD320* KO brain tissue than in control.

TCbIR has an essential role in ensuring vitamin B<sub>12</sub> bioavailability. Transcobalamin, the plasma vitamin B<sub>12</sub> transporter, saturated with vitamin B<sub>12</sub> binds to the TCbIR, and is internalized by endocytosis [15]. The TCbIR/*CD320* KO mice did not express functional TCbIR,



so cellular vitamin B<sub>12</sub> uptake by this route was not possible. As a result, vitamin B<sub>12</sub> depletion occurred in TCbIR/CD320 KO mouse brain but not in control brain that had normal TCbIR expression. However, this gene knockout is not embryonic lethal and so far it appears that the mice appear to breed normally.

Choi, *et al*, demonstrated that vitamin B<sub>12</sub> depletion in rats, following ten weeks on a vitamin B<sub>12</sub>-deficient diet, induced a 35% reduction in genomic DNA methylation of rat colonic epithelium compared with control rats [11]. The results from our study support this observation. We found that in the TCbIR/CD320 KO mice, with



depleted vitamin B<sub>12</sub> in the brain, global DNA methylation was 44% lower than in control vitamin B<sub>12</sub> replete mice.

Methylcobalamin is a cofactor for MS, responsible for methyl transfer from homocysteine to methionine [16]. This metabolic pathway is impaired when vitamin B<sub>12</sub> supply is low. As a result, SAH accumulates and this affects the ability of DNA methyltransferase enzymes to methylate cytosine residues and DNA hypomethylation occurs [17].

Vitamin B<sub>12</sub> deficiency leads to defective myelin formation [9] and epigenetic factors, such as histone modifications, are involved in this process [18]. The change in DNA methylation pattern observed in the TCbIR/CD320 KO mouse brain might contribute to the demyelination process that occurs in advanced vitamin B<sub>12</sub> deficiency. To confirm this hypothesis, the methylation pattern of genes involved in the myelination process and its association with changes in their gene expression should be investigated.

This preliminary study provides evidence of severe impairment in DNA methylation capacity in the brain of TCbIR/CD320 KO mice.

#### Abbreviations

MS: Methionine synthase; SAM: S-adenosylmethionine; TCbIR: Transcobalamin receptor; KO: Knock out; CNS: Central nervous system.

#### Competing interests

EVQ is an inventor in a patent (WO2007/117657 A2) filed by the Research Foundation of SUNY. Other authors have no conflicts of interest.

#### Acknowledgements

Supported by NIH grant R01DK064732 to EVQ. SFR received a predoctoral grant from the URV and her stay in Prof Quadros' lab was financed by CIBEROBN: Fisiopatología de la Obesidad y Nutrición (CB06/03) (Instituto de Salud Carlos III), becas de movilidad del personal de investigación.

#### Author details

<sup>1</sup>Area of Preventive Medicine and Public Health, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili (URV), IISPV, Reus, Spain. <sup>2</sup>CIBER Fisiopatología de la Obesidad y Nutrición (CB06/03) Instituto Carlos III, Madrid 28029, Spain. <sup>3</sup>Departments of Medicine and Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY, USA. <sup>4</sup>Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA.

#### Author's contributions

EQ: Principal investigator of the study and responsible for generating the hypothesis, EQ, SFR and SCL: Participated in the design and coordination of the study, SFR, SCL, EQ, JDFB, MM: Responsible for writing and statistical analysis, EQ, SCL, SFR, JDFB, MM: were involved in the analysis of the data. All authors read and approved the final version of the manuscript.

#### Availability of supporting data

NA

Received: 22 March 2012 Accepted: 18 May 2012

Published: 18 May 2012

#### References

1. Selhub J: Folate, vitamin B<sub>12</sub> and vitamin B<sub>6</sub> and one carbon metabolism. *J Nutr Health Aging* 2002, **6**:39–42.

2. Hsu DW, Lin MJ, Lee TL, Wen SC, Chen X, Shen CK: **Two major forms of DNA (cytosine-5) methyltransferase in human somatic tissues.** *Proc Natl Acad Sci U S A* 1999, **96**:9751–9756.
3. Wolffe AP, Matzke MA: **Epigenetics: regulation through repression.** *Science* 1999, **286**:481–486.
4. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP: **Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription.** *Nat Genet* 1998, **19**:187–191.
5. Eden A, Gaudet F, Waghmare A, Jaenisch R: **Chromosomal instability and tumors promoted by DNA hypomethylation.** *Science* 2003, **300**:455.
6. Laird PW, Jaenisch R: **The role of DNA methylation in cancer genetic and epigenetics.** *Annu Rev Genet* 1996, **30**:441–464.
7. Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ: **Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation.** *J Biol Chem* 2000, **275**:29318–29323.
8. Reynolds E: **Vitamin B12, folic acid, and the nervous system.** *Lancet Neurol* 2006, **5**:949–960.
9. Miller A, Korem M, Almog R, Galboiz Y: **Vitamin B12, demyelination, remyelination and repair in multiple sclerosis.** *J Neurol Sci* 2005, **233**: 93–97.
10. Piyathilake CJ, Johanning GL, Macaluso M, Whiteside M, Oelschlager DK, Heimbürger DC, Grizzle WE: **Localized folate and vitamin B-12 deficiency in squamous cell lung cancer is associated with global DNA hypomethylation.** *Nutr Cancer* 2000, **37**:99–107.
11. Choi SW, Friso S, Ghandour H, Bagley PJ, Selhub J, Mason JB: **Vitamin B-12 deficiency induces anomalies of base substitution and methylation in the DNA of rat colonic epithelium.** *J Nutr* 2004, **134**:750–755.
12. Chen X, Guo J, Lei Y, Zou J, Lu X, Bao Y, Wu L, Wu J, Zheng X, Shen Y, Wu BL, Zhang T: **Global DNA hypomethylation is associated with NTD-affected pregnancy: A case-control study.** *Birth Defects Res A Clin Mol Teratol* 2010, **88**:575–581.
13. Quadros EV: **Vitamin B<sub>12</sub>.** In *Modern Analytical Methodologies in Fat- and Water-Soluble Vitamins*. Edited by Song WO, Beecher R, Eitenmiller RR. New York: John Wiley & Sons, Inc; 2000:313–328.
14. User Guide: **MethylFlash Methylated DNA Quantification Kit (Colorimetric).** [<http://www.epigentek.com/catalog/methylflash-methylated-dna-quantification-kit-colorimetric-p-2094.html>].
15. Youngdahl-Turner P, Mellman IS, Allen RH, Rosenberg LE: **Protein mediated vitamin uptake, Adsorptive endocytosis of the transcobalamin II-cobalamin complex by cultured human fibroblasts.** *Exp Cell Res* 1979, **118**:127–134.
16. Markle HV: **Cobalamin.** *Crit Rev Clin Lab Sci* 1996, **33**:247–356.
17. Ross SA, Poirier L: **Proceedings of the Trans-HHS Workshop: diet, DNA methylation processes and health.** *J Nutr* 2002, **132**:2329S–2332S.
18. Shen S, Li J, Casaccia-Bonnel P: **Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain.** *J Cell Biol* 2005, **169**:577–589.

doi:10.1186/1743-7075-9-41

**Cite this article as:** Fernández-Roig *et al.*: Vitamin B<sub>12</sub> deficiency in the brain leads to DNA hypomethylation in the TCbIR/CD320 knockout mouse. *Nutrition & Metabolism* 2012 **9**:41.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

