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Transcobalamin receptor gene polymorphisms and mutation in an elderly population

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SUMMARY

Background & aims: Cellular uptake of the essential nutrient vitamin B12 (cobalamin) occurs via the transcobalamin receptor (TCblR/*CD320*), a ubiquitous membrane receptor. Polymorphisms in the receptor exist, though the effect of such variants across patient populations is unknown.
Methods: We determined *CD320* genotype in 377 randomly selected elderly individuals.
Results: Three polymorphisms and a codon deletion were identified in the exon 2 region. Haplotype variants had significantly higher holotranscobalamin (holo-TC) values and a higher holo-TC/total cobalamin ratio. TCblR haplotype explained 46% of the variability in holo-TC values.
Conclusions: This has significant implications for the clinical utility of the 'combined indicator' of B12 status since it is based on a standard rate of intracellular flux via the TC-Cbl receptor. Modification of the model may be required to account for *CD320* haplotype.

1. Introduction

Vitamin B_{12} (cobalamin) is an essential nutrient required for DNA synthesis and fatty acid metabolism [1]. It plays a key role in the maturation of red blood cells and the maintenance of nervous system structure and function. Its biological importance is reflected in the detailed sequence of events concerning its assimilation. Following ingestion, it is released from food and becomes bound to salivary haptocorrin [2]. Pancreatic proteases then degrade haptocorrin in the duodenum releasing free B_{12}, which is subsequently bound to intrinsic factor (IF). After ileal absorption by the IF receptor cubilin, the IF-B_{12} complex is degraded in lysosomes and B_{12} is released into the circulation. Here it is associated with two separate blood carriers: haptocorrin and transcobalamin. However, only transcobalamin (TC-Cbl) delivers the vitamin to cells. The final step in its assimilation occurs via cellular uptake of this complex, which is mediated by the transcobalamin receptor (TCblR/*CD320*) [3].

Variants of the *CD320* gene locus [19p13] are known to exist. A homozygous single codon deletion (NM_016579.3:c.262_264delGAG (p.Glu88del) was first reported in 2010 in a new-born infant with methylmalonic acidaemia [4]. Cobalamin was elevated in the index case, suggesting reduced cellular uptake by the receptor. In fact, this variant results in a two-fold decrease in affinity for TC-Cbl. Similarly, in large cohorts of healthy younger and older adults, a heterozygous deletion is significantly associated with increased circulating holo-TC concentration at the genome-wide level [5].

The life-long effects of such a deletion in the elderly population perhaps modify cobalamin homeostasis and could have important clinical relevance. For example, moderately elevated plasma total homocysteine (tHcy), a marker of cobalamin and folate status, is now recognized as a significant modifiable risk factor for dementia, including Alzheimer's disease [6,7]. The relative risk of dementia in elderly people for moderately raised tHcy ranges from 1.15 to 2.5, and it is estimated that almost one-third of dementia cases might be attributable to tHcy [6]. Furthermore, intervention trials show

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that tHcy-lowering treatment with B vitamins markedly slows brain shrinkage *and* cognitive decline in elderly individuals with cognitive impairment [8–10]. Moreover, a Bayesian network analysis suggests that cobalamin is the key determinant in this relationship [10,11]. Therefore, we sought to identify common variants of *CD320* and determine their effects on cobalamin status in an aging population.

2. Materials and methods

The individuals studied were participants in the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) cohort (www.cfas.ac.uk). This is a longitudinal multicenter study of community dwelling elderly persons aged 65 years and older living in either their own home or residential accommodation who were randomly selected from population-based registers in six United Kingdom sites [12]. As part of MRC CFAS blood samples were collected in 1997 in accordance with the European Prospective Investigation of Cancer guidelines [13]. A 30-mL sample of blood was collected in EDTA-coated, citrate-coated, or plain bottles, and couriered overnight to a central laboratory prior to separation and storage at −80 °C as separate aliquots of serum, plasma, red cells, and buffy coats. A randomly selected subset of 377 individuals had measures of serum cobalamin, holo-TC, and methylmalonic acid (MMA) determined in 2008 and these were used to investigate the TCblR gene. Genomic DNA from whole blood was analysed by amplicon DNA sequencing of the exon 2 region for all individuals. MMA was measured by Gas Chromatography-Mass Spectrometry and holo-TC by the Axis-Shield active B_{12} immunoassay which provides a measure of the fraction of TC that is saturated with cobalamin and is available for cellular uptake.

2.1. Statistical considerations

Continuous variables are presented as means with their 95% confidence intervals and discrete variables presented as a percentage. The chi squared test was used for comparisons between discrete variables and the Kruskal Wallis test for comparisons between continuous variables. A linear regression model was used to assess the contribution of age, gender and CD320 haplotype to the variability of serum B12 and holo-TC values. Analysis of genotype data to derive values for i) Hardy–Weinberg equilibrium for each variant and ii) linkage disequilibrium between variants was undertaken using PLINK software [14].

Ethical statement

At its outset in 1991 the MRC Cognitive Function and Ageing Study (CFAS) was approved locally at all sites. Following the introduction of Multi-centre Research Ethics Committees CFAS applied for both Multi-centre and Local Research Ethics Committee approval at each centre (See https://www.cfas.ac.uk/files/2015/07/Ethical-approvals-for-CFAS.pdf). The Research Ethics Committees changed the protocol titles many times over the years but the overall goal and consent to analyse samples remained unchanged.

3. Results

The mean age of subjects was 79 years. 53% were female. Cobalamin deficiency was highly prevalent; 28% had holo-TC< 37 pmol/L and 53% had MMA above the upper reference interval of 0.42 μmol/L. Four polymorphisms within the exon 2 region of the TCblR (*CD320*) gene were identified: an intron 3 T > G SNP (rs2232780) (MAF = 0.058); a heterozygous GAG deletion causing a glutamic acid deletion at codon 88 (MAF = 0.026); an intron 3 G > A SNP (rs2232781) (MAF<0.01), and a novel A > G SNP encoding a p. T61A amino acid substitution (MAF<0.01).

Haplotype analysis of the most common polymorphism (rs2232780 (T > G) and a GAG deletion was undertaken. In 377 individuals the haplotype frequencies were as follows: WT/WT (n = 335), WT/rs2232780 G (n = 22) and rs2232780 G/GAG deletion (n = 20). There was a high level of linkage disequilibrium between rs2232780 and the GAG deletion (D' = 1.0, R^2 = 0.44). MMA did not differ by haplotype, although variants had significantly higher holo-TC values and a higher holo-TC/Cbl ratio (p < 0.001) (Table 1). In a regression model, age, gender and *CD320* haplotype explained 46% of the variability in holo-TC values with *CD320* variation accounting for a significant amount of this (p < 0.001) (Table 2).

4. Discussion

We found that TCblR/*CD320* polymorphisms exist in an elderly population. We limited our analysis to the exon 2 region and identified that as many as 12% of elderly individuals carry a

Table 1
Demographics and metabolites associated with polymorphisms/deletion in exon 2 region of *CD320* gene.

	WT/WT (n = 335)	WT/rs2232780 G (n = 22)	rs2232780 G/GAG del (n = 20)	• Discrete Data – Chi square; Continuous Data – Kruskal Wallis Test Statistic; Probability under H_0
Age	79 (78.5, 80)	80 (77, 83.5)	79 (76, 82)	0.289; p = 0.87
Sex (% Female)[a]	52	55	70	2.49; p = 0.29
MMA	0.57 (0.53, 0.61)	0.62 (0.37, 0.87)	0.52 (0.31, 0.73)	2.25; p = 0.32
Creatinine	98 (94, 101)	102 (89, 116)	90 (80, 100)	1.16; p = 0.56
Serum Folate	5.5 (5.1, 5.9)	4.1 (3.3, 4.9)	5.1 (3.3, 6.9)	2.86; p = 0.24
Serum B_{12}	215 (198, 231)	183 (150, 215)	174 (143, 204)	1.60; p = 0.45
Holo-TC	**54 (50, 56)**	**66 (54, 79)**	**80 (63, 96)**	**15.57; p = < 0.001**
Holo-TC/B_{12} (%)[a]	**27 (26, 28)**	**37 (31, 43)**	**46 (40, 51)**	**43.78; p = < 0.001**

[a] Data: mean (95% ci) or %.

Table 2
Influence of haplotype frequency on B_{12} and holo-TC.

Variable	R^2	Age	Women	WT/rs2232780 G	rs2232780 G/GAG del
Serum B_{12}	3%	3.3 (1.1–5.5) P = 0.004	−0.9 (−30.6 – 28.8) P = 0.96	−34.6 (−97.5-28.3) P = 0.28	−40.3 (−106.2-25.7) P = 0.23
Holo-TC	46%	−0.5 (−0.9–−0.2) P = 0.002	6.3 (1.9–10.7) P = 0.005	17.6 (8.3–27) P = 0.001	30.4 (20.5–40.2) P = 0.001

haplotype containing at least one of these polymorphisms. Furthermore, the presence of a polymorphism is associated with proportionately more serum cobalamin bound as holo-TC and, together with age and gender, TCblR/*CD320* haplotype explains 46% of holo-TC's variability.

There is no recognized 'gold standard' for determining B_{12} status. There are four laboratory biological markers of B_{12} deficiency: total serum B_{12}, holo-TC, plasma tHcy and MMA. However, each has its limitations [15]. Total serum B_{12} is most frequently used, but these assays mainly provide a measure of B_{12} bound to haptocorrin which accounts for about 80% of serum cobalamin and not available for cellular uptake. The assays are also influenced by the presence of interfering substances such as anti-IF antibodies in patients with pernicious anaemia and lack the precision and sensitivity to detect changes in holoTC which accounts for <30% of serum cobalamin [16].

The two 'functional' indicators of B_{12} status – plasma tHcy and MMA - are based on the rationale that insufficiency of the vitamin impairs the activity of two B_{12}-dependent enzymes, namely methionine synthase and methylmalonic acid Co-A mutase, causing respective accumulation of their substrates. Although these can provide a better insight into an individual's B_{12} status, the lack of consensus for their cut-off values remains problematic in diagnosing B_{12} deficiency [15]. Furthermore, tHcy levels also reflect folate status, and MMA levels are influenced by renal function. Both can also be affected by thyroid function status.

An alternative suggestion is to use holo-TC to evaluate B_{12} status, though it remains uncertain whether this is any better than the measurement of serum B_{12} itself [17]. However, holo-TC does appear to be unaffected by assay interference from high-titre IF antibody levels [18].

If the results from the above markers of B_{12} status are ambiguous Fedosov et al. suggest using a 'combined indicator' of B_{12} status [19]. This is a rigorously derived mathematical model combining all four markers and is adjusted for age and folate status. It yields one of five diagnoses: elevated B_{12}, adequate B_{12}, decreased B_{12}, possibly B_{12}-deficient, and probably B_{12}-deficient. Its main drawbacks are the associated expense and general lack of availability of all these markers within routine clinical practice, though the authors have recently provided a refined model that provides for 'missing markers' [20].

However, our results suggest that holo-TC is perhaps not necessarily a marker of 'true' vitamin B_{12} status in all patients. In certain individuals, 'high' holo-TC levels might also reflect decreased cellular uptake due to an inherited specific *CD320* haplotype. This observation has significant clinical implications for the 'combined indicator' of B_{12} status, since this model is based on an assumed standard rate of intracellular flux via the TC-Cbl receptor [19,20]. Some modification of the model is likely to be required to account for an individual's known *CD320* haplotype. Further larger confirmatory studies are required. It will also be interesting to evaluate the effect of *CD320* haplotype on the incidence of disorders associated with B_{12} insufficiency in the elderly, such as cognitive decline and dementia, including Alzheimer's Disease [21].

Statement of authorship

AM conceived the study, DFC and PH performed statistical analyses, SM determined serum cobalamin, holo-TC and MMA assays and EVQ performed DNA sequencing of TCblR/*CD320*. All authors contributed equally to writing the paper.

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Declaration of competing interest

No author had any conflict of interest to declare.

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