Short Communication

Common variant in FUT2 gene is associated with levels of vitamin B_{12} in Indian population

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A B S T R A C T

Vitamin B_{12} is an essential micronutrient synthesized by microorganisms. Mammals including humans have evolved ways for transport and absorption of this vitamin. Deficiency of vitamin B_{12} (either due to low intake or polymorphism in genes involved in absorption and intracellular transport of this vitamin) has been associated with various complex diseases. Genome-wide association studies have recently identified several common single nucleotide polymorphisms (SNPs) in fucosyl transferase 2 gene (FUT2) to be associated with levels of vitamin B_{12}—the strongest association was with a non-synonymous SNP rs602662 in this gene. In the present study, we attempted to replicate the association of this SNP (rs602662) in an Indian population since a significant proportion has been reported to have low levels of vitamin B_{12} in this population. A total of 1146 individuals were genotyped for this SNP using a single base extension method and association with levels of vitamin B_{12} was assessed in these individuals. Regression analysis was performed to analyze the association considering various confounding factors like for age, sex, diet, hypertension, diabetes mellitus and coronary artery disease status. We found that the SNP rs602662 was significantly associated with the levels of vitamin B_{12} (p value < 0.0001). We also found that individuals adhering to a vegetarian diet with GG (homozygous major genotype) have significantly lower levels of vitamin B_{12} in these individuals. Thus, our study reveals that vegetarian diet along with polymorphism in the FUT2 gene may contribute significantly to the high prevalence of vitamin B_{12} deficiency in India.

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1. Introduction

Vitamin B_{12}, also known as cobalamin, is an essential micronutrient that is primarily involved in the catalysis of two biochemical reactions—conversion of methylmalonyl CoA to succinyl CoA where adenosyl cobalamin acts as a cofactor of the enzyme methionine synthase. In humans, vitamin B_{12} is obtained from food source or is synthesized by microorganisms in the gut (Albert et al., 1980). Its absorption, transport and cellular uptake in the body is carried out by three different proteins—haptocorrin (HC), intrinsic factor (IF) and Transcobalamin II (TCII) (Quadros et al., 1999; Seetharam, 1999). Vitamin B_{12} is involved in various critical processes like formation of red blood cells, DNA synthesis and maintenance of the myelin nerve sheath (Reynolds, 2006; Weir and Scott, 1999). Deficiency of this vitamin is associated with several disease conditions like megaloblastic anemia, impaired immune defense, gastrointestinal and neurological disorders (Clarke et al., 1998; Hall and Finkler, 1966; Kang et al., 2006; Sellhaub et al., 2009). Recently, we have shown that in the Indian population, low levels of vitamin B_{12} are associated with coronary artery disease (CAD) (Kumar et al., 2009). Further, deficiency of vitamin B_{12} leads to elevated levels of homocysteine, a condition called hyperhomocysteinemia that is considered to be an independent risk factor for cardiovascular diseases (Splaver et al., 2004). Hyperhomocysteinemia is also associated with various other complex disorders like neural tube defects, Alzheimer’s disease, end-stage renal disease, schizophrenia, non-insulin-dependent diabetes etc. (McCully, 2007; Mills et al., 1995; Robinson et al., 1995; Schwartz et al., 1997; Splaver et al., 2004; Zhang et al., 2003).

Deficiency of vitamin B_{12} could arise due to its low intake. This micronutrient is usually found in abundance in animal products like fish, meat and poultry (Watanabe, 2007) and is practically absent in plant products. Thus, it is not surprising that several studies including ours
have clearly shown that the level of vitamin B₁₂ is significantly low in individuals adhering to a strict vegetarian diet. Further, defects in absorption, transport or uptake of this micronutrient may also lead to deficiency of vitamin B₁₂. For instance, in elderly, absorption of protein bound vitamin B₁₂ is decreased as compared to a younger population presumably due to atrophic gastritis resulting in low acid-pepsin secretion which leads to lower release of free vitamin B₁₂ (Baik and Russell, 1999). Moreover, several single nucleotide polymorphisms (SNPs) have also been reported to be associated with levels of vitamin B₁₂ (Haggarty, 2007). Although some of these SNPs have been replicated in various populations, others are not (Garg et al., 2012; Garrod et al., 2010; Kumar et al., 2010; Stanislawski-Sachadyn et al., 2010). Most of these SNPs were selected based on candidate gene approach (Fredriksen et al., 2007; Haggarty, 2007). However, with the advent of high density genotyping arrays, it is now possible to hunt for SNPs that are associated with a disease or a quantitative trait using an unbiased genome-wide association study (GWAS) approach. Recently, using this approach, Tanaka et al. have identified a few SNPs that are associated with levels of vitamin B₁₂ (Tanaka et al., 2009). The top loci were replicated in another study and it was found that a SNP in fucosyl transferase (FUT2) gene (G772A, rs6022662) is associated with the levels of plasma vitamin B₁₂. In a separate study, Hazra et al. carried out GWAS on women of European ancestry and have shown that SNPs in FUT2 gene (rs602662, rs601338 and rs492602) are associated with levels of vitamin B₁₂ (Hazra et al., 2008). Further, a meta-analysis of three GWAS on 4763 individuals of European ancestry for one carbon metabolite revealed that all three SNPs in FUT2 gene are associated with levels of vitamin B₁₂ (Hazra et al., 2009).

From various GWAS studies, the SNP in FUT2 gene (rs602662) has been consistently shown to be the most significant SNP associated with levels of vitamin B₁₂. Although association of this SNP has been replicated in several Caucasian populations, there has been no study evaluating the association in the Indian population despite the fact that a large number of individuals in this population are deficient in vitamin B₁₂ (Kumar et al., 2009). Thus, we have undertaken this study to analyze the association of SNP rs602662 with levels of vitamin B₁₂ in the Indian population.

2. Material and methods

2.1. Subjects

A total of 1146 individuals belonging to an Indo-European linguistic group mainly from North India were included in this study. This was a part of a study aimed at evaluating the SNPs that are associated with CAD and its risk factors. Out of these 1146 individuals, 461 were angiographically confirmed CAD patients and 424 were treadmill test negative controls collected from the Department of Cardiology, All India Institute of Medical Sciences, New Delhi, India. The rest 261 were healthy controls recruited from the general population. Written informed consent was obtained from each participant prior to their recruitment in the study. Blood samples were collected and a questionnaire was filled on the day of recruitment that included information on diet, smoking, height, weight, disease history etc. Pregnant women or individuals below 18 years of age were excluded from the study. All the experiments were performed within the guidelines of the ethical committee of participating institutes.

2.2. Blood collection and biochemical parameters

10 ml venous blood sample was drawn from each individual. For serum separation, plain tubes were used and serum was isolated within an hour and stored at −80 °C until further used. Genomic DNA was isolated from blood cells using a modified salting out method explained elsewhere (Kumar et al., 2005) and stored in −20 °C until further use. Levels of vitamin B₁₂ were determined by electro-chemiluminescence immunoassay using ELECSYS 2010 immunoassay analyzer (Roche Diagnosis, USA) as per the manufacturer’s instructions. The ELECSYS vitamin B₁₂ assay is based on a competitive test principle using intrinsic factor specific for vitamin B₁₂. Vitamin B₁₂ in the sample competes with the added vitamin B₁₂ labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex. Vitamin B₁₂ levels were determined by quantification of ruthenium-labeled intrinsic factor-vitamin B₁₂ biotin complex.

2.3. PCR and genotyping

Flanking regions of the SNP rs602662 were obtained from NCBI (http://www.ncbi.nlm.nih.gov). Oligonucleotides for amplification and genotyping were synthesized in house. Oligonucleotide sequences are given in Supplementary Table 1. It was ensured that the synthesized oligonucleotides were not complimentary to any other region in the genome.

Genomic DNA was amplified using polymerase chain reaction or PCR (GeneAmp® PCRSystem 9700, Applied Biosystems, Foster City, USA); PCR optimizations were carried out using different concentrations of MgCl₂ and annealing temperatures for an oligonucleotide pair. Each PCR reaction was performed in a 10 µl reaction mixture, containing 20 ng genomic DNA. The PCR conditions used were: initial denaturation for 5 min at 95 °C followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 5 s followed by a final extension at 72 °C for 10 min. The amplified product was then purified by a polyethylene glycol purification method (Rosenthal et al., 1993). A single base primer extension method was utilized for genotyping using a ddNTP extension assay (SNAPSHOT kit™, Applied Biosystems, Foster City, CA, USA). Genotypes were scored with the help of ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Kumar et al., 2005).

2.4. Statistical analysis

Genotypic and allelic frequencies were calculated using the standard method. Genotyping was tested for the conformation of Hardy–Weinberg equilibrium (HWE) using a chi-square test. Since the measured levels of vitamin B₁₂ did not follow normal distribution, a non-parametric test was employed to assess the association with an SNP. Linear regression analysis was performed to analyze the association of the SNP with levels of vitamin B₁₂ and various confounding factors like age, sex, diet, hypertension, diabetes mellitus and CAD status were adjusted. A p-value < 0.01 was considered to be significant. The data was analyzed employing the statistical program SPSS version 17.0 (SPSS, Chicago, IL, USA). To detect a variance of 1%, we had 87% statistical power at alpha 0.01 in our total samples.

3. Results

The general/clinical characteristics of the studied population are shown in Table 1. Among the samples included in this study, 48.2% (n = 553) were vegetarians. The median levels of vitamin B₁₂ in vegetarians and non-vegetarians were 145.4 pmol/l and 159 pmol/l respectively.

The genotypic distribution and allele frequencies of the studied SNP are shown in Table 2. The genotypic distribution followed the HWE (p > 0.01). In this population, allele A was found to be minor with a minor allele frequency (MAF) of 0.31 which was comparable to that reported in dbSNP (Table 2). The AA genotype (homozygous minor) was present in only 10.8% of the individuals studied. When the median levels of vitamin B₁₂ among different genotypic groups were compared, individuals with the homozygous major genotype (GG) had the lowest levels (149.5 pmol/l). Individuals with the AA genotype had the highest median levels of vitamin B₁₂ (175.3 pmol/l).
followed by the heterozygous genotype (GA, 152.7 pmol/l). To analyze the association of SNP rs602662 with levels of vitamin B12, we carried out linear regression analysis taking vitamin B12 as a dependent variable and adjusted for various confounding factors like age, sex, diet, hypertension, diabetes mellitus and CAD. We found that this SNP was significantly associated with levels of vitamin B12 (p = 4.0 × 10⁻⁵, Table 3).

Since a significant proportion of the studied population are vegetarians and are expected to be deficient in vitamin B12, the analysis of association for the SNP rs602662 in FUT2 gene with levels of vitamin B12 was performed separately also for vegetarians and non-vegetarians. We found that vegetarians with the GG and GA genotypes had significantly lower levels of vitamin B12 compared to nonvegetarians while those with the AA genotype had comparable levels of this vitamin (Table 3).

4. Discussion

In this study, we have replicated the association of the SNP rs602662 in FUT2 gene with levels of vitamin B12. Various GWAS had earlier shown that common variants in FUT2 gene are significantly associated with levels of vitamin B12. The strongest association was with a non-synonymous SNP rs602662 (Hazra et al., 2009; Tanaka et al., 2009). This SNP is located in exon 2 of the FUT2 gene and it has been reported that the presence of an A allele is associated with a 44.2 pg/ml higher concentration of vitamin B12. Thus, carriers of the A allele will have higher levels of vitamin B12 than the carriers of the G allele which is consistent with our observations as well. Hazra et. al reported a frequency of the G allele to be 0.49 in the Caucasian population studied. However, the frequency of this allele in our population was found to be 0.69 and that of the A allele as 0.31. This indicated that a greater number of individuals in our population carry the G allele and hence is expected to have lower levels of vitamin B12.

FUT2 gene codes for a secretor enzyme α-1,2-fucosyltransferase and is responsible for the transfer of fucose to form a terminal H type 1 structure (Kelly et al., 1995; Watkins, 1980). The SNP rs602662 is reported to be in linkage disequilibrium with nonsense SNP rs601338 (W143X) that is believed to be the causal variant which is characteristic for the nonsecretor allele. Individuals with the homozygous major genotype for this SNP are mostly nonsecretors having inactive FUT2.

Interestingly, in a study involving individuals from Northern Portugal, Serpa et al. have shown that mutants of FUT2 rs601662 G-A SNP is almost inactive for the fucosylation activity (Serpa et al., 2004). It has been reported that pathogens such as Helicobacter pylori (H. pylori) attach themselves to either α,1,2-fucosylated glycans or to precursor structures masked by fucosylation on epithelial cells (Boren et al., 1993). Thus, secretors with an active FUT2 enzyme are at greater risk of infections from such pathogens. FUT2 secretor status has been associated with H. pylori infection and gastritis (Dickey et al., 1993). Infections with H. pylori in human intestine have been reported to affect the absorption of vitamin B12 (Carmel et al., 1994; Kaptan et al., 2000). It has been shown that SNPs in genes affecting the susceptibility of such microbes result in mal-absorption of vitamin B12 (Carmel et al., 1987). Infection with H. pylori is common among Indians due to poor socioeconomic status. H. pylori infection increases with age, as its frequency of occurrence in the age group 3–10 years is 60%, while at the age of 20 years it increases to more than 80% in the general population (Gill et al., 1994; Graham et al., 1991). A hospital based study from Delhi, India reported a seroprevalence of 52% by 20 years of age and peak prevalence of 68% before 40 years of age (Jais and Barua, 2004). Thus, it can be perceived that individuals with an SNP at FUT2 gene will be susceptible to H. pylori infection and hence have reduced secretion of intrinsic factor leading to reduced levels of vitamin B12.

Our study was done in a population where a significant proportion of individuals adhere to a strict vegetarian diet throughout their life mainly due to religious compulsions. It is therefore not surprising that a large proportion of the Indian population have low levels of vitamin B12. Reports from various parts of this country have projected that 30–60% of the population irrespective of the age group are deficient in vitamin B12 (plasma levels < 150 pmol/l) (Refsum et al., 2001). Deficiency of this vitamin ranges from 14 to 40% in Indian children in the age group of 3 months to 18 years (Gomber et al., 1998, 2003; Hanumante et al., 2008; Sivakumar et al., 2006; Taneja et al., 2007), 43–77% in pregnant women and women of child bearing age (21–24 years) (Krishnaveni et al., 2009; Pathak et al., 2007; Yajnik et al., 2005, 2008). Interestingly, the average levels of vitamin B12 even in non-vegetarians in India are considerably lower as compared with other populations (Kumar et al., 2009). Our results also indicate that vegetarians with the GG genotype have significantly lower vitamin B12 levels than non-vegetarians with the same genotype indicating a possibility of a combined effect of diet and genotype responsible for the high prevalence of vitamin B12 deficiency in the Indian population. It is therefore very relevant to study the effects of genetic polymorphisms associated with vitamin B12 in this population.

5. Conclusions

In conclusion, we have been able to replicate the association of rs602662 in FUT2 gene in the Indian population. We also show that individuals adhering to a vegetarian diet with GG (homozygous major

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**Table 1**

General clinical characteristics of the studied individuals.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 1146)</th>
<th>VEG (n = 553)</th>
<th>NON-VEG (n = 593)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49 (40–57)</td>
<td>50 (41–59)</td>
<td>47 (37–55)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (21.5–27.2)</td>
<td>24.5 (22.0–27.3)</td>
<td>24.6 (22.0–27.3)</td>
<td>0.622</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/l)</td>
<td>154.2 (114.6–208.4)</td>
<td>145.4 (110.0–195.3)</td>
<td>159.0 (121.4–217.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt;150 pmol/l (n [%])</td>
<td>542 (47.3)</td>
<td>288 (52.1)</td>
<td>254 (42.8)</td>
<td></td>
</tr>
<tr>
<td>Female [n (%)]</td>
<td>240 (20.9)</td>
<td>139 (25.1)</td>
<td>–101 (17.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>DM [n [%]]</td>
<td>178 (15.5)</td>
<td>–93 (16.8)</td>
<td>85 (14.3)</td>
<td>0.230</td>
</tr>
<tr>
<td>Hypertension [n [%]]</td>
<td>325 (28.4)</td>
<td>147 (26.6)</td>
<td>–178 (30.0)</td>
<td>0.218</td>
</tr>
</tbody>
</table>

n = number of individuals, median (interquartile range) reported for continuous variables while number (percentage) reported for discrete variables. VEG—vegetarians, NON-VEG—non-vegetarians; DM—diabetes mellitus.

* P-value = calculated using chi-square or Mann–Whitney test. VEG and NON-VEG groups were compared.

**Table 2**

Genotypic distribution and allele frequencies of the FUT2 rs602662 polymorphism.

<table>
<thead>
<tr>
<th>N</th>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Total</td>
<td>1146</td>
<td>558 (48.7)</td>
</tr>
<tr>
<td>VEG</td>
<td>553</td>
<td>252 (45.6)</td>
</tr>
<tr>
<td>NON-VEG</td>
<td>593</td>
<td>306 (51.6)</td>
</tr>
</tbody>
</table>
Table 3
Genotypic distribution of FUT2 rs602662 polymorphism with levels of vitamin B12.

<table>
<thead>
<tr>
<th>Genotypic group</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
<th>Standardized coefficient</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>140.4 (110.7–197.8)</td>
<td>152.6 (115.6–211.1)</td>
<td>175.3 (120.6–270.9)</td>
<td>0.120</td>
<td>4.0 × 10⁻³</td>
</tr>
<tr>
<td>VEG</td>
<td>140.7 (106.3–193.5)</td>
<td>146.5 (110.7–191.7)</td>
<td>176.4 (124.3–240.4)</td>
<td>0.116</td>
<td>5.0 × 10⁻³</td>
</tr>
<tr>
<td>NON-VEG</td>
<td>156.3 (116.6–205.8)</td>
<td>160.7 (125.5–228.8)</td>
<td>174.2 (118.1–288.8)</td>
<td>0.120</td>
<td>4.0 × 10⁻³</td>
</tr>
</tbody>
</table>

Median (interquartile range) is reported. VEG—vegetarians, NON-VEG—non-vegetarians.

* Linear regression was performed adjusting for age, sex, diet, hypertension, diabetes mellitus and CAD status. In VEG and NON-VEG, linear regression was performed adjusting for age, sex, hypertension, diabetes mellitus and CAD status.

genotype) have significantly lower levels of vitamin B12 in this population.

Conflict of interest statement

The authors report no conflict of interest.

Role of the funding source

The funding agency provided the required funds to perform the study without any influence on study design or results.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2012.11.021.

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