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Duodenal expression of the epithelial calcium transporter gene *TRPV6*: is there evidence for Vitamin D-dependence in humans?☆

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Abstract

Intestinal absorption of dietary calcium is regulated by 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) in humans and in experimental animals but interspecies differences in responsiveness to $1,25(OH)_2D_3$ are found, possibly due to differences in the promoters of genes for intestinal calcium transport proteins or of the Vitamin D receptor (VDR). The epithelial calcium transporter, known as ECAC2 or CAT1, the product of the *TRPV6* gene expressed in proximal intestinal enterocytes, is the first step in calcium absorption and studies in mice have shown that its expression is Vitamin D-dependent. In contrast in man, we showed that duodenal TRPV6 mRNA expression was independent of blood $1,25(OH)_2D_3$, although in Caco-2 cells, $1,25(OH)_2D_3$-dependent changes have been demonstrated. We sought to explain these findings. A consensus Vitamin D response element in the mouse gene is absent in the human gene. We re-analysed our duodenal expression data according to a CDX2-site polymorphism in the VDR promoter. Mean TRPV6 expression was the same, but there was evidence of different responsiveness to $1,25(OH)_2D_3$. In the *GG* genotype group, but not the *AG*, duodenal TRPV6 expression increased with $1,25(OH)_2D_3$. We postulate that lower levels of expression of VDR in the *GG* group produce greater sensitivity to $1,25(OH)_2D_3$.

Keywords: Intestine; Calcium absorption; Human; TRPV6; $1,25(OH)_2D_3$; Vitamin D receptor; Single nucleotide polymorphism; Vitamin D response element

1. Introduction

The ability to absorb calcium varies greatly in healthy people: individual fractional calcium absorption values between 10 and 50% are found, and such differences persist over time [1,2]. Subjects with low fractional calcium absorption who also had low calcium intake have a significantly higher incidence of osteoporotic fractures [3]. Surprisingly, the reasons for the variation in the ability to absorb calcium remain largely unexplained, and hence dietary calcium requirements and the need for supplementation are not individualised. In multiple regression studies, groups have shown that the variability in fractional calcium absorption is related in part to factors such as intestinal transit, dietary fat and fibre, urinary excretion and to Vitamin D metabolites [4,5]. However, only a minor proportion of the variability is explainable by these factors. Additionally, the effects of ageing, intestinal resistance to Vitamin D, smoking, dietary calcium, birth weight and certain polymorphisms in the Vitamin D receptor may all produce some effect. The proportion of the variation in fractional calcium absorption that is dependent on blood levels of $1,25(OH)_2D_3$ is surprisingly small, less than 25%, as shown by correlation coefficients of less than 0.5. [5–9].

What other factors could determine the fraction of dietary calcium absorbed? In previous work [10,11], we showed that the expression in the intestine of specific calcium transport molecules (calbindin-D9k, PMCA1b and TRPV6) differs greatly in individuals. Understanding the regulation of expression of these genes will predict the ability to absorb calcium, enabling more targeted nutritional advice to be given regarding calcium intake and supplements.

The key transporter appears to be the first step in transcellular calcium transport, the recently described epithelial apical membrane calcium channel previously known as ECAC2 or CAT1, the product of the *TRPV6* gene. TRPV6 is one of two closely related molecules first described in 1999 [12,13]. Studies have looked at the molecular properties of these channels and have confirmed that they are functional, selective calcium entry channels. TRPV6 is expressed in human proximal intestine; we showed there is a wide 10-fold variation in transcript levels in duodenal biopsies from normal individuals [11].

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In this published data we found no correlation between human TRPV6 and plasma $1,25(OH)_2D_3$; despite strong associations with the expression of this RNA with those for calbindin-D9k and PMCA1, the calcium transporters in the cytoplasm and basolateral membrane. In mice, however, there is good evidence for regulation of TRPV6 by $1,25(OH)_2D_3$. Knockouts of the genes for either the Vitamin D receptor or the $1,25(OH)_2D_3$ synthetic enzyme 1α-hydroxylase, dramatically reduced both calcium absorption and TRPV6 expression [14–16]. Changes in dietary calcium levels in the mouse also result in changes in TRPV6 expression, partly through PTH regulation of 1α-hydroxylase. The human Caco-2 colonic cell-lines have provided evidence that there may be $1,25(OH)_2D_3$-responsiveness of human TRPV6 [17,18].

We have looked for possible explanations for these divergent findings. One reason for interspecies differences in the responsiveness of the intestinal calcium transport proteins to $1,25(OH)_2D_3$ may be due to differences in Vitamin D response elements (VDRE) in their promoters. Another factor which could influence these results is differences in the expression of components of the Vitamin D endocrine system, including the Vitamin D receptor (VDR). The *VDR* gene has several polymorphic areas. A single nucleotide polymorphism (SNP) has been identified in the 1e promoter of the *VDR* gene in a consensus response element for the caudal-related homeobox transcription factor CDX2, highly expressed in intestine [19]. This CDX2-site SNP has been shown to affect transcription of the *VDR* gene and to be associated with differences in bone mineral density and fracture risk [20,21]. We hypothesised that this SNP could result in differences in intestinal VDR expression which could then affect the expression of Vitamin D-dependent genes, including *TRPV6*, involved in calcium absorption.

1.1. Methods

Human *TRPV6* gene sequence was obtained by from a human BAC gridded genomic library. The VDR SNP at the CDX2-site in the promoter was determined by PCR amplification using primers described before [20]. The genotype (*GG*, *AG* or *AA*) was determined by sequencing. DNA was used from 82 patients in West London with known bone mineral density and from 32 patients where intestinal gene expression had previously been studied [10,11].

2. Results

2.1. TRPV6 promoters

To look for differences in $1,25(OH)_2D_3$ responsiveness of TRPV6 expression between man and mouse we compared the sequence of the respective gene promoters. The human *TRPV6* gene sequence obtained from our sequencing of a genomic clone was confirmed by sequence in the Human Genome Project (AC104597). We looked for consensus transcription factor response elements, including VDRE, in 940 bases of 5′-flanking region using the MatInspector program (Geomatrix). This was compared with the mouse *Trpv6* promoter from AF336378. A consensus VDRE at about −150 from the translation initiation site in the mouse gene (AGGTGA agt AGGAGA) is deleted in the human gene. No other VDRE is apparent but it is possible to identify potential VDRE sequences in the human gene which fit the consensus less well. Although several SNPs are apparent in the human gene, it is not polymorphic in this region.

2.2. CDX2-site VDR polymorphism

The genotypes determined in our British population were as follows: $GG = 49$, $AG = 27$ and $AA = 6$. The observed allelic frequency was $G = 76\%$, $A = 24\%$, with a lower proportion of A alleles than in the Japanese population ($G = 57\%$, $A = 43\%$) [20], but similar to other European studies [21]. No significant difference could be detected between the different genotype groups in the proportion of patients with normal BMD, osteopenia or osteoporosis. However these analyses are under-powered to detect changes in BMD similar to those described by others.

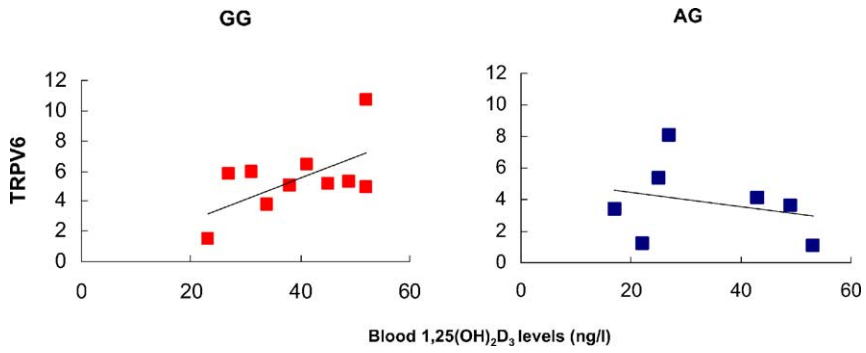

Fig. 1. Relationship between duodenal TRPV6 mRNA and plasma $1,25(OH)_2D_3$ according to the *VDR* CDX2-site genotype. Numbers of individuals: $GG = 10$; $AG = 7$. Different relationships are found. *GG*: $y = 0.14x$; $r^2 = 0.33$; $F = 4.00$; $P = 0.08$. *AG*: $y = -0.05x + 5.41$; $r^2 = 0.08$; $F = 0.41$; $P = 0.55$.

2.3. Duodenal gene expression and CDX2-site genotype

Further analysis was made of mRNA expression in duodenum in *GG* and *AG* groups. There were no significant differences in mean values of TRPV6, calbindin-D9k or PMCA1. However, evidence was found for different responsiveness to $1,25(OH)_2D_3$ in these two groups. Fig. 1 shows the relationship between duodenal TRPV6 expression and $1,25(OH)_2D_3$. In the *GG* group, a positive association is found between $1,25(OH)_2D_3$ and TRPV6, which approaches significance ($r = 0.57$, $P = 0.08$, $n = 10$). No association is present in the *AG* group—that is they show no $1,25(OH)_2D_3$-dependence in TRPV6 expression. The relationship of calbindin-D9k expression and $1,25(OH)_2D_3$ was also stronger in the *GG* than in the *AG* group.

3. Discussion

Despite not being able to demonstrate a conserved VDRE in 940 bp of the 5′-flanking region of the human *TRPV6* gene, these results suggest that in a subgroup of individuals, $1,25(OH)_2D_3$-dependent duodenal TRPV6 expression may be found. This would then support the findings in model human cell-culture systems.

A possible mechanism that explains these findings is as follows. Individuals with the *GG* genotype, if the *A* allele is dominant, can be predicted to have lower VDR expression in intestinal cells. The *A* allele of VDR promoter has higher affinity for CDX2 and produces greater reporter expression in model systems [20]. Greater levels of expression of VDR occur in those with *A* allele and hence, a larger number of occupied receptors will be found at any $1,25(OH)_2D_3$ concentration. Expression may then be less dependent on levels of $1,25(OH)_2D_3$. Data from Caco-2 cells supports this, with clones with higher levels of VDR expression being less responsive to $1,25(OH)_2D_3$. [18]. Measurement of duodenal levels of VDR in groups with the different genotypes would test this hypothesis.

We conclude that the $1,25(OH)_2D_3$-dependence of the human *TRPV6* gene may only be apparent in subgroups of the population when other variable factors in the Vitamin D endocrine system are taken into account. These other factors may play important roles in the individual variability of calcium absorption, the response to low calcium diets, the risk of bone disease and the need for dietary supplements.

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