EXPRESSION OF HUMAN CALBINDIN-D_{9K} GENE IN INTESTINE

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Introduction

The Calbindin-D_{9k} (CaBP-9K), is a cytosolic protein found in intestine, placenta and uterus of mammalian[1, 2]. The function of CaBP-9K is possibly involved in the process of calcium transfer in intestine, placenta, and epithelium of uterus. The regulation of CaBP-9K in various tissues is not completely understood. Whereas the intestinal levels are clearly vitamin D controlled, placental and uterine concentrations do not respond to vitamin D-depletion administration. The CaBP-9K gene contains the Vitamin D-responsive DNA element (VDRE) and this element is responsible for its expression in intestine[5]. Especially, this gene is only active in the enterocyte, the major epithelial cell of the duodenal mucosa[3], and its expression level decrease to downstream from duodenum, and barely found in the digital ileum and large intestine[4]. In the enterocyte, Ca^{2+} enter through the epithelial calcium channel from the lumen, the Ca^{2+} is bound to the calcium binding protein CaBP-9K, and the Ca^{2+} is discharged to the blood by the plasma membrane calcium ATPase PMCA1. The CaBP-9K has been thought to be one of important factors of calcium absorption and metabolism in the intestine.

The aim of this study is to examine the level of the human CaBP-9K and VDR mRNA in intestine with aging and gender whether there is any relationship with mineral homeostasis and risk for the development of age-related calcium disorders such as osteoporosis.

Methods and Materials

Normal gastrointestinal tissues of antrum of stomach, bulb and 2nd portion of duodenum were obtained from 39 healthy outpatients by a routine upper-gastrointestinal endoscorpy. The tissues were extracted from the patient within three hours of inspection. The tissues were requested to be flash frozen in liquid nitrogen to prevent degradation of the RNA by RNase. Total RNA was prepared from biopsies, according to a protocol for single-step RNA isolation based on acid guanidinium-thiocyanate-phenol-chloroform extraction, using TRIzol reagent. After denaturation, 10 μg total RNA samples were submitted to electrophoresis in 1% formaldehyde-agarose denaturing gel, transferred to nylon membranes and hybridized with human CaBP-9K probe. The probe was labeled with $\alpha[^{32}P]$-

dCTP by a random-primed DNA labeling procedure with Klenow polymerase. Hybridization of the membrane was carried out and exposed to ray films.

Complementary DNA sequences for the detection of mRNA transcripts of VDR, CaBP-9K, and IA were obtained by RT-PCR of intestinal RNA extract using appropriate primers and reverse transcriptase enzyme. This gel was denatured and blotted onto the nylon membrane. Hybridization of the membrane carried out with cDNA template obtained by RT-PCR and VDR primers and expose to X-ray films. The signals of the Northern and Southern autoradiograms were quantified using a personal scanner and quantified using Molecular Program. Results of the densitometric determinations of VDR and CaBP-9K mRNA were corrected according to the corresponding IA and 18S, respectively. The statistical analysis was performed by student's T-test.

Results and Discussion

We measured intestinal CaBP-9K mRNA on humans of different ages and gender in an attempt to shed some light on a possible molecular mechanisms underlying the well known differences in intestinal calcium absorption between young and old age. Figure 1 shows the results of CaBP-9K mRNA expression in antrum of stomach, bulb and 2nd portion of duodenum, measured by Northern blot analysis, in both gender humans aged thirty to seventy years. As expected, we found a significant expression of CaBP-9K mRNA in the bulb and 2nd portion of duodenum over age in both men and women. The expression level of duodenal CaBP-9K increased with age significantly in both men and women. However, no significant differences of CaBP-9K mRNA expression were found between men and women. The findings of Wood *et al.*[3] which indicated correlation of CaBP-9K expression with gender, are not easy to reconcile with the effect of gender on the CaBP-9K mRNA expression. It would also have been of interest to measure serum estradiol level to see whether there was any relationship with gene expression.. Surprisingly, however, we observed no significant age-associated reduction in intestinal CaBP-9K mRNA concentration, as others have reported in rat model.

As seen in Figure 2, the level of VDR mRNA expression is mainly detected in both stomach and duodenum. The variation in VDR expression between subjects analyzed was considerable. The abundance of VDR mRNA in duodenum followed a similar pattern to that of CaBP-9K mRNA in stomach and intestine over age in both men and women. However, we observed no significant age-associated increase in VDR mRNA level in the duodenum. Our studies of CaBP-9K content in the duodenum did not reveal an expected age-associated positive correlation in intestinal vitamin D receptor mRNA as others have reported.

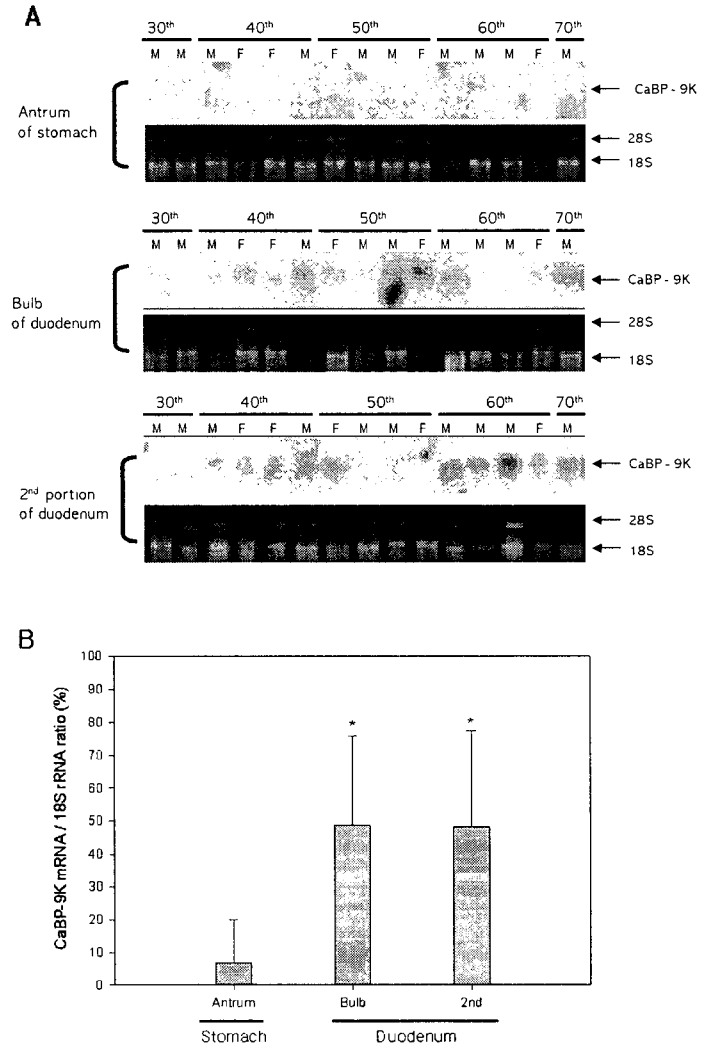

Figure 1. Result of a Northern blot analysis (A) of RNA (10 μg/lane) from human antrum (stomach), bulb (duodenum), and 2nd portion (duodenum) with age (30, 40, 50, 60 and 70th) probed with ^{32}P-labeled human CaBP-9K RACE clone and exposed for two days. The lower panel (B) summarizes the densitometric analysis of replicate experiments (mean±SEM of values expressed as a percentage of CaBP-9K/18S rRNA, n= 39). Statistical analysis was performed by student's t-test. *: Significantly different compared with antrum at $P<0.001$.

Waters *et al.*[3] showed a similar degree of correlation of the amounts of expressed calcium binding protein in human duodenum.

As expected, plasma calcium concentrations decreased with age in both men and women. No gender difference was found for plasma calcium concentrations (data not shown). Our study found no correlation overall between calcium level and CaBP-9K expression in human. However, the findings

of Waters *et al.*, which indicated correlation of the CaBP-9K with calcium absorption with age, are not easy to reconcile with VDR affecting CaBP-9K transcription. Many changes that occurred with aging in human of both genders were as expected. In this study we have shown that duodenal biopsies demonstrated a positive correlation with VDR mRNA expression in the population.

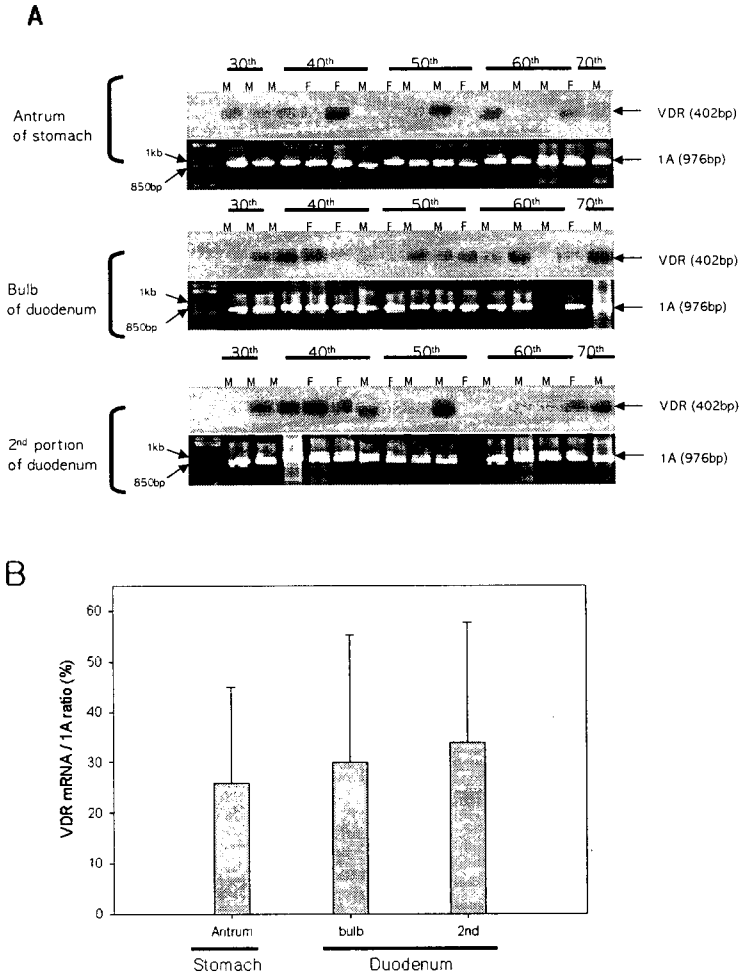

Figure 2. The upper panel (A) is a representative of RT-PCR and southern hybridization analysis for VDR expression in antrum (stomach), bulb (duodenum), and 2nd portion (duodenum) with age (30, 40, 50, 60 and 70th). The lower panel (B) summarizes the densitometric analysis of replicate experiments (mean ± SEM of values expressed as a percentage of VDR/ IA mRNA, n= 39). Statistical analysis was performed by student's T-test. No significant changes in the VDR expression were detected ($P < 0.05$).

Acknowledgments

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