Interaction between CFTR and Cl^-/HCO_3^- Exchanger SLC26A6 is Essential for HCO_3^- Secretion in the Intestine

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Background: Approximately 30,000 people in America are affected by cystic fibrosis. In this autosomal recessive disorder, in which a mutant form of the cystic fibrosis transmembrane conductance regulator (CFTR) becomes inactivated, there are multiple clinical manifestations, the most severe being thickening of the mucous of the lung and inability to produce or deliver bicarbonate and enzymes in the digestive system. CFTR is an apical anion channel that mediates secretion of Cl^- into the lumen from the cell, and subsequently drives HCO_3^- secretion via activation of Cl^-/HCO_3^- exchange. SLC26A6 is the primary apical chloride/bicarbonate exchanger in the small intestine, and is also present on the apical membrane of the pancreatic duct and the kidney proximal tubule. It has been previously suggested that SLC26A6 and CFTR are linked through their PDZ binding domain.

Objectives:

1) Confirm that both CFTR and SLC26A6 are located on the apical membranes of enterocytes in the mouse and rat duodenum.
2) Describe the interaction between CFTR and SLC26A6, and show that they are physically bound to one another.

Methods: Tissue sections of mouse and rat duodenum were prepared and single immunofluorescent labeling with either CFTR or SLC26A6 specific antibodies was performed. Separately, rat duodenum enterocytes were obtained, lysed, and then immunoprecipitated with CFTR antibodies. This immunoprecipitate was then analyzed using Western blot with antibodies for SLC26A6. In addition, the supernatant from the immunoprecipitation reaction was also analyzed with Western blot and antibodies for SLC26A6 to see if SLC26A6 was present.

Results: Sections of both mouse and rat duodenum displayed fluorescent labeling on the apical surface of enterocytes when either SLC26A6 or CFTR antibodies were used. The immunoprecipitation from rat enterocytes and subsequent Western blot of the immunoprecipitate yielded a band at approximately 70kD. This band correlates to the location of the SLC26A6 protein. The supernatant yielded faint lines in the 70 kD range.

Conclusions and Significance: With both CFTR and SLC26A6 located on the apical membrane of enterocytes, these results demonstrate physical interaction between the two transport proteins. This conclusion is based on the above results indicating that CFTR causes the immunoprecipitation of SLC26A6 protein, which was verified by Western blot analysis using SLC26A6 antibodies. These results strongly suggest that CFTR and SLC26A6 are linked and bound together. If there is similar binding between these two proteins in humans, as in the rat duodenum, and SLC26A6 is dependent on this interaction with CFTR, then this may provide an explanation for the bicarbonate secretion defect in various epithelia in cystic fibrosis patients when a mutant protein may not allow binding to SLC26A6.

Reference values for bone mass and density of the lumbar spine for children 6 to 36 months of age.

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Dual-energy x-ray absorptiometry (DXA) is widely used for measuring bone mineral content (BMC) and density (BMD) to aid the assessment of bone health in children. Currently, there are no reference values of bone mass and density for children 6 to 36 months of age that can be used as a standard for comparison. The aims of this project were: 1) to recruit 200 healthy children 6 to 36 months of age (100 boys, 100 girls); 2) to determine if there are age and sex differences in BMC and BMD of the lumbar spine; and 3) to develop age-specific BMC and BMD reference values based on current generation DXA technology. Eligibility criteria were weight and length between the 5^{th} to 95^{th} percentiles for age, normal gross motor skill attainment, and absence of health conditions known to affect BMC. A DXA scan of the lumbar spine was obtained by a Hologic QDR4500A densitometer, and analyzed by software version 12.4 to give measurements of bone area, BMC and BMD. A total of 52 subjects were recruited (27 girls, 25 boys), a DXA scan was not obtained on 4 subjects because of intense crying, and a "usable" DXA scan (no movement) was obtained on 37 subjects. The likelihood of a "usable" scan increased with age: 57% of infants 6-18 months of age vs. 88% of infants 18-36 months of age. Bone area, BMC and BMD increased linearly with age ($P<0.001$). Bone area and BMC were smaller in girls than in boys ($p< 0.001$), but there was no sex difference in BMD. Reference ranges for bone measures should be developed separately for boys and girls and should be age specific. The utility of developing reference DXA data for infants < 18 months of age is questionable.