To the editor:

Cubilin and the hydrophobic intrinsic factor receptor are distinct molecules

Cubilin is a 460-kd multiligand hydrophobic protein that binds to intrinsic factor cobalamin (Cbl-IF) with a high affinity and that is expressed in both kidney and ileal epithelial cells. Based on this observation, it has been speculated that cubilin and intrinsic factor cobalamin receptor (IFCR) are identical. Recently, Kristiansen et al showed that the P1297L mutation of cubilin (FM1) associated with selective congenital cobalamin malabsorption (Imerslund-Gräsbek disease, described in Finnish patients; also named megaloblastic anemia 1 [MGA1]) causes a 5-fold decrease of the binding domain affinity for Cbl-IF. They concluded that this decreased affinity explained the malabsorption of Cbl and the Cbl deficiency related to the FM1 mutation of MGA1 patients. In our opinion, the pathogenetic mechanism suggested by Kristiansen et al does not explain the whole biologic phenotype of the Finnish patients. The P1297L mutation of cubilin has been found in 16 of 17 Finnish patients but not in patients from Norway or Kuwait. It has been located in the same 6-cM region of chromosome 10 as the disease locus identified by linkage analysis by Aminoff et al in Norwegian and Finnish patients. We have found 4 French cases of Imerslund-Gräsbek disease that the binding activity of a hydrophobic IFCR was dramatically decreased in both ileum and urine, using a radioisotopic assay in which \([^{57}\text{Co}]\text{Cbl-IF}\) bound to the receptor was precipitated by adsorption to phenyl-Sepharose.

In affected patients, the affinity of the IFCR for Cbl-IF was similar to that found in the ileums and the urine of nonaffected healthy subjects. In collaboration with M. Aminoff, the study was extended to 10 Finnish patients, 11 first-degree relatives and 13 healthy control subjects. We confirmed our previous finding, with a dramatic decrease of the IFCR activity in all the urine specimens (640 times lower than in control urine), whereas the affinity of IFCR for Cbl-IF remained unmodified, with a \(K_d\) ranging from 1.2 to 4.8 (nmol/L)\(^{-1}\) in patients and from 1.4 to 6.4 (nmol/L)\(^{-1}\) in controls. Unlike what was found for the hydrophobic IFCR, Aminoff et al have detected mutated cubilin by Western blot of urine in affected Finnish patients, with the same intensity and molecular size as that in controls, and Kristiansen et al have shown now that the affinity of the mutated protein for Cbl-IF was decreased 5-fold. The opposite behavior of cubilin and IFCR in urines with regard to the excretion level and the Cbl-IF affinity suggests, therefore, that cubilin and the hydrophobic IFCR are distinct molecules. In a previous study, Moestrup et al showed that cubilin binds to other proteins such as apoA-I, albumin, light chains of IgG, and RAP (receptor-associated protein) in domains other than the Cub 5-8 IF-Cbl binding domain. They concluded that the multiligand activity of cubilin may explain the proteinuria observed in some of the affected patients. Considering that the P1297L mutation of cubilin affects only its binding to IF\(^2\) and has no consequences on its binding activity to other proteins,\(^3\) this does not explain why 40% of the Finnish series had proteinuria while none of them, except a case with another mutation, had an excretion of apoA-I in urine. The binding of cubilin to the IFCR-Cbl-IF complex may be a molecular event needed for the endocytosis of the complex. The IFCR and cubilin have a distinct intracellular distribution. Indeed, intracellular cubilin is located in the apical dense tubules,\(^7\) whereas the IFCR-IF-Cbl complex is targeted to the lysosomal pathway. Both molecules bind Cbl-IF with the same affinity and a similar pH-dependent pattern. But cubilin is weakly bound to the membrane via its N-terminal amphipathic domain, as it can be released by ethylenediaminetetraacetic acid (EDTA) washing. In contrast, the 70-kd IFCR is very hydrophobic, and EDTA washing increases activity rather than removing it.\(^5\) In addition, the molecule purified from hog intestine can be integrated through a phospholipid bilayer.\(^10\) Taken together, these data suggest that the hydrophobic IFCR has a transmembrane domain. The suggestion we made 2 years ago that cubilin and IFCR may be distinct molecules is reinforced by the report by Xu et al that the schnauzer dog model of MGA1 is not caused by a mutation of the CUBN gene nor by any gene within 50 cM of either side of the CUBN locus.\(^11\) In conclusion, the discovery by Moestrup and coworkers that cubilin has an IF-Cbl binding activity by cubilin and its implication in Imerslund-Gräsbek disease represents a very significative and convincing advance of our knowledge of Cbl cellular transport. But it may be assumed that the pathogenetic concept discussed by Kristiansen et al is only part of the mechanism and that cubilin and the hydrophobic IFCR are distinct molecules that may act in concert.

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References


Response:

Evidence for an accessory activity required for cubilin expression—not for an alternative intrinsic factor cobalamin receptor

We appreciate that Dr Gueant recognizes the importance of our scientific work on the intrinsic factor cobalamin (IF-Cbl) receptor, cubilin. But it is very difficult for us to find scientific support for several of his statements, including the argument for the existence of a distinct 70-kd IF-Cbl receptor and the hypothesis that such a distinct protein should be affected in Finnish patients with Imerslund-Gräsbeck (MGA1) having the P1297L cubilin mutation causing Cbl deficiency.

In several papers by the groups of Seetharam,1,2 Fyfe,3 Verroust,4,6 and us,4,6 it has been definitely proven that the 460-kd cubilin protein is identical to the high molecular IF-Cbl receptor (IFCR) protein. None of these groups or other groups have ever reported the existence of an alternative 70-kd receptor (as Dr Guéant also designates IFCR). The introduction of the paper by Guéant and colleagues7 on the 70-kd IF-Cbl–binding urinary protein contains the following statements about the protein: “It is probably produced by proteolytic cleavage of the receptor for IF-cobalamin located in the epithelium of the kidney tubules. This urinary receptor is likely to be a part of the same protein expressed in the ileum.”7(p25) Because of the high proteolytic activity in the urine, we believe in this possibility and we can confirm (unpublished data, November 1997) that cubilin released from the tubules is partially degraded in the urine. Furthermore, we have shown that CUB domains 5-8 of cubilin constitute the IF-Cbl binding region of cubilin.8 The size of this region is about 65-70 kd (glycosylated) and has a theoretical isoelectric point (amino acids only) of 5.4, which is close to the estimated 4.8 value measured for the reported 70-kd protein in the urine. It is therefore a great surprise for us that Dr Guéant now argues that his reported 70-kd protein should represent a distinct IF-Cbl receptor. It would be relevant to determine the primary structure of the 70-kd protein, or at least a part of it. In this way it would ultimately be elucidated whether the 70-kd protein and cubilin are distinct proteins or not. If the 70-kd protein is distinct from cubilin, it should be characterized in terms of structure and function.

Dr Guéant claims that the low IF-Cbl binding activities that he has measured in the urine of Finnish patients with Imerslund-Gräsbeck are “opposite behavior” to our data on the affinity decrease caused by the P1297L mutation. We do not agree. First, several of his statements, including the argument for the existence of a distinct 70-kd IF-Cbl receptor and the hypothesis that such a distinct protein should be affected in Finnish patients with Imerslund-Gräsbeck (MGA1) having the P1297L cubilin mutation causing Cbl deficiency.

Finally, Dr Guéant claims that a study10 on a canine Imerslund-Gräsbeck disease model reinforces his theory that cubilin and the putative 70-kd protein are distinct molecules. However, the conclusion of this interesting study by Xu et al10 is not that there exists an alternative IF-Cbl receptor but that these affected dogs have a genetic defect in an accessory activity important for cubilin processing. The genetic data of the Xu et al paper10 are very convincing, and in our recent Blood paper11 we discuss the possibility that patients with Imerslund-Gräsbeck in ethnic populations other than the Finnish might have a genetic defect similar to that of the dogs.

References

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