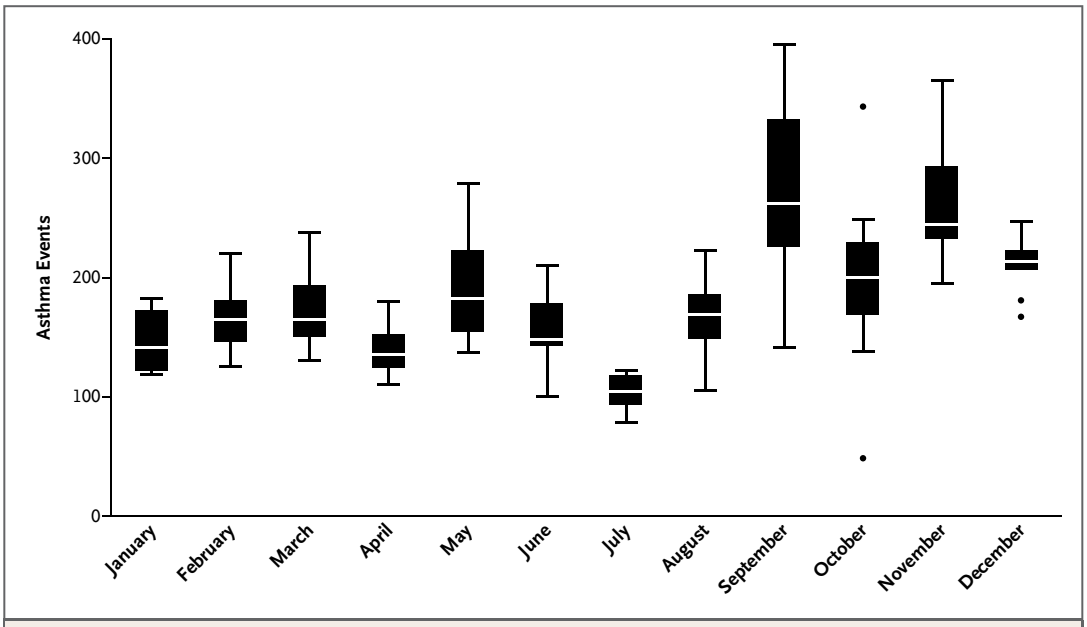

Figure 1. Hospital Admissions for Childhood Asthma in Scotland between January 2000 and October 2009, According to Month.

Boxes indicate the interquartile ranges; the lines within the boxes are medians. The vertical lines represent 1.5 times the interquartile range (above the upper end of the interquartile range and below the lower end of the interquartile range). The dots indicate the lowest and highest values.

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Since publication of their article, the authors report no further potential conflict of interest.

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A Patient with Cubilin Deficiency

TO THE EDITOR: Imerslund–Gräsbeck syndrome, or megaloblastic anemia 1, is a rare autosomal recessive disorder characterized by selective intestinal malabsorption of intrinsic factor–vitamin B_{12}; it is frequently accompanied by tubular proteinuria.[1] The syndrome is caused by mutations in the genes encoding the receptor partners cubilin (*CUBN*) or amnionless (*AMN*),[2] both of which are highly expressed in the absorptive epithelia of the ileum and the proximal tubules of the kidney. Cubilin, which interacts in the proximal tubules with megalin, another receptor with a high molecular weight, is critical to receptor-mediated tubular reabsorption of several important ligands from glomerular ultrafiltrate.[3]

We describe here a patient with a novel homozygous guanine-for-thymine exchange in the conserved donor splice site in exon 23 of *CUBN* (see Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Tests performed on a renal-biopsy specimen showed no immunologic reaction for cubilin and an abnormal cytoplasmic, vesicular distribution of the receptor partner amnionless (Fig. 1), indicating that amnionless depends on cubilin for correct localization in the human

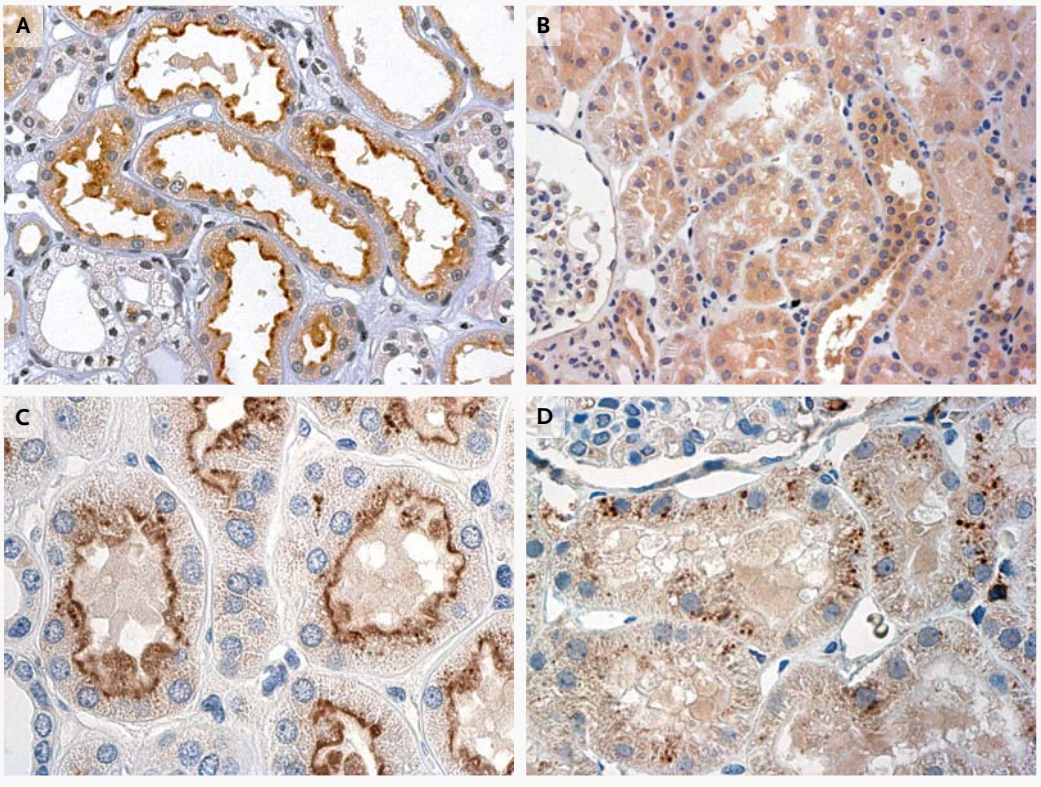

Figure 1. Renal-Biopsy Specimens from a Patient with Imerslund–Gräsbeck Syndrome and from a Healthy Subject.
Staining of a tissue sample from a healthy subject was positive for cubilin (Panel A), whereas cubilin was not detected in a tissue sample from a patient with Imerslund–Gräsbeck syndrome (Panel B) (immunoperoxidase staining with the use of a polyclonal murine autoantibody against cubilin; titer, 1:500). (The specimen from the patient had normal immunoreactivity for megalin; see Fig. S2 in the Supplementary Appendix.) Staining for amnionless in a specimen from a healthy subject showed normal apical, proximal tubular localization for amnionless (Panel C), whereas a stained sample from the patient with Imerslund–Gräsbeck syndrome showed an abnormal, cytoplasmic, vesicular distribution of amnionless (Panel D) (immunoperoxidase staining with the use of a polyclonal human autoantibody against amnionless; titer, 1:400).

proximal tubule. The interdependency of cubilin and amnionless has previously been analyzed in a spontaneous Imerslund–Gräsbeck canine model, in which the *AMN* homologue was mutated.[4] In this model, cubilin had an abnormal, cytoplasmic, vesicular distribution — the inverse of our patient's situation. In contrast, previous renal histologic analyses performed with specimens from patients with Imerslund–Gräsbeck syndrome suggested only minor, unclear morphologic changes[5] (see the Supplementary Appendix). Megalin distribution was unaffected, and its endocytic function was normal (Fig. S2 in the Supplementary Appendix).

Apolipoprotein A-I, a cubilin ligand, was not detected in our patient's kidney, although there was normal vesicular staining for a vitamin D–binding protein (a ligand shared by cubilin and megalin). Together with increased urinary excretion of apolipoprotein A-I and transferrin (both cubilin ligands; see Fig. S3A in the Supplementary Appendix), the fact that apolipoprotein A-I could not be detected in the kidney indicates the coexistence of selective cubilin dysfunction and maintained megalin endocytic function (Fig. S4A through S4D in the Supplementary Appendix). This is further supported by the increased urinary excretion of vitamin D–binding protein (a shared ligand) and the lack of urinary excretion of retinol-binding protein or β_2-microglobulin (megalin ligands).

Increased urinary excretion of α_1-microglob-

ulin (a putative megalin ligand) was also detected in this patient, but immunohistochemical analyses revealed no apparent change in its uptake (Fig. S4E and S4F in the Supplementary Appendix), which suggested β_2-microblobin is also a shared ligand. Consequently, we tested the binding capacity of α_1-microglobulin to cubilin (with the use of surface-plasmon-resonance analyses, as described in the Supplementary Appendix and shown in Fig. S3B) and found that α_1-microglobulin is also a cubilin ligand.

Studies in rodents have shown that both cubilin and amnionless are essential to normal embryonic development. However, no lethal phenotype or malformations were observed in the dogs deficient in amnionless in the above-mentioned model. Since our patient has no additional apparent developmental abnormalities or physical disabilities, it appears that cubilin is not essential for human embryonic development. Furthermore, the functional immunohistochemical analyses of specimens from this patient's kidney indicate that cubilin and amnionless also have an interdependent relationship in humans.

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A *SOX9* Duplication and Familial 46,XX Developmental Testicular Disorder

TO THE EDITOR: Female-to-male sex reversal in humans is rare, and when it is familial, it is extremely rare. We describe a family with a 46,XX testicular disorder of sex development in which three adult males (two brothers and a paternal uncle) were determined to be female according to karyotype (46,XX) and were negative for the *SRY* gene (Fig. 1). The secondary sexual characteristics, behavior, growth and development, and skeletal development in these men were all normal male. Their general health and intelligence were normal. All three affected men were infertile with azoospermia. In two men, the testes were removed and prostheses were placed during their 20s because of testicular pain secondary to testosterone replacement. Histologic examination showed the presence of Leydig and Sertoli cells, severely diminished and atrophied seminiferous tubules, and no spermatogenesis.

Male development is normally triggered by the transient expression of the Y chromosome gene *SRY*, which initiates a cascade of gene interactions orchestrated by *SOX9*, leading to the formation of testes from bipotential gonads.[1,2] The essential and nonredundant role of *Sox9* in male development was initially detected in mice. Ectopic expression of *Sox9* in the female gonad of XX mice caused complete female-to-male sex reversal, demonstrating that *Sox9* is sufficient to trigger testis differentiation in the absence of *Sry*.[3,4]