Leading article

The molecular and genetic base of congenital transport defects

In the past 10 years, several monogenetic abnormalities have been identified in families with congenital intestinal transport defects. Wright and colleagues^{1,2} described the first, which concerns congenital glucose and galactose malabsorption. Subsequently, altered genes were identified in partial or total loss of nutrient absorption, including cystinuria, lysinuric protein intolerance, Menkes' disease (copper malabsorption), bile salt malabsorption, certain forms of lipid malabsorption, and congenital chloride diarrhoea. Altered genes may also result in decreased secretion (for chloride in cystic fibrosis) or increased absorption (for sodium in Liddle's syndrome or copper in Wilson's disease)—for general review see Scriver and colleagues,³ Desjeux,⁴ and Krawczak and Cooper⁵ (<http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html>). When considering the rarity of these diseases, we may ask why gastroenterologists should be concerned with these discoveries? My personal answer is that we may gain information in three main areas: (1) the pathophysiology and treatment of these diseases; (2) the use of genetics in gastroenterology; and (3) the genetic control of nutrient absorption. Thus by recognising the entry of genetics into the field of gastroenterology, we may have to adapt to a new way of thinking to fully participate in restoring or maintaining the good health of mankind. In this paper, for the sake of simplicity, only monogenic diseases will be considered.

Relationship between phenotype and genotype

In some diseases the relationship between phenotype and genotype is quite convincing. Glucose and galactose malabsorption (GGM) is a rare congenital disease resulting from a selective defect in the intestinal glucose and galactose/Na⁺ cotransport system. It is characterised by neonatal onset of severe watery acidic diarrhoea. Children recover if glucose and galactose are withdrawn from the diet. Interestingly, GGM was described by French and Swedish paediatricians when the American biochemist R K Crane was presenting his hypothesis on the glucose-Na⁺ cotransporter at the luminal membrane of the enterocyte, and the American physiologists and biophysicists S G Schultz and P F Curran were discovering the relationship between water, sodium, and glucose absorption. All the ingredients were present to suggest that it was a genetic disease but identification of the genetic alteration came almost 30 years later.⁶

Following the cloning of the gene SGLT1, coding for the human glucose-Na⁺ cotransporter, it was possible to investigate the molecular defect in GGM. The location of SGLT1 was refined to 22 q 13.1. It belongs to the solute carrier family 5; accordingly its gene symbol is SLC5A1.

Given the size of SGLT1 mRNA (2.3 kb), the gene is large, with 15 exons, and the introns range between 3 and 2.2 kb. A single base change was identified in the entire coding region of one child, a finding that was confirmed in the other afflicted sister. This was a homozygous guanine to adenine base change at position 92. The patient's parents were heterozygotes for this mutation. In addition, it was found that the 92 mutation was associated with inhibition of sugar transport by the protein. Since the first familial study, genomic DNA has been screened in 31 symptomatic GGM patients in 27 kindred from different parts of the world. In all 33 cases the mutation produced truncated or mutant proteins. In the case of missense mutation, the defect is caused by improper trafficking of the protein to the luminal membrane of the enterocyte or malfunction of the protein, or both. Homozygous mutations were observed in 60%, in agreement with the autosomal recessive nature of the disease.²

Thus the discovery of the gene coding for the glucose and galactose-Na⁺ cotransport system at the luminal membrane of the enterocyte together with the finding that its alteration in families with the disease is associated with a functional loss of the transporter, clearly indicates the genetic nature of the disease; it explains its pathophysiology and the efficacy of glucose removal from the diet.

In dibasic (or diamino) amino acid transport defects the situation is more complex. Lysine, arginine, ornithine, and cystine are the four amino acids found in the urine of cystinuric patients; the first three are also characteristic of hyperdibasic aminoaciduria, and isolated cases of cystinuria or lysinuria have been reported. In addition, genetic heterogeneity may be present in the first two diseases and the defects may not be identical in the kidney and intestine. Therefore, genetic control for the transport of this group of amino acids must be at four different sites at least.³

Cystinuria is the most common congenital amino acid transport defect. The symptoms are exclusively those of urinary tract stones because of poor solubility of cystine at the usual urinary pH. There are no gastrointestinal symptoms or signs. In contrast, lysinuric protein intolerance (LPI) is characterised by renal dibasic aminoaciduria, especially massive lysinuria, and inadequate formation of urea with hyperammonaemia after protein ingestion. It is a life threatening disease.

Abbreviations used in this paper: GGM, glucose and galactose malabsorption; LPI, lysinuric protein intolerance; CFTR, cystic fibrosis transmembrane conductance regulator; CLD, congenital chloride diarrhoea; DRA, down regulated in adenoma; IFM, isolated fructose malabsorption;

Cystinuria is inherited as an autosomal recessive trait. All homozygotes have identical urinary excretion patterns and abnormalities in renal clearance, but the pattern of intestinal transport defects and the amount of abnormal urinary excretion in heterozygotes suggests at least three different mutations. In type I cystinuria characterised by absence of aminoaciduria, mutations have been found at the SLC3A1 gene coding for the cystine, dibasic, and neutral amino acid transporter. In type III, homozygotes show a near normal increase in cystine plasma levels after oral cystine administration. In types II and III, heterozygotes show high or moderate excretion of cystine and dibasic amino acids. They do not cosegregate with the SLC3A1 gene.⁷

LPI is inherited as an autosomal recessive trait in Finland; one new case is diagnosed among 60 000 to 80 000 births. Heterozygotes are not detectable. Mutations in the SLC7A7 gene that codes for the dibasic (or cationic) amino acid transporter (or y (+) LAT-1 system) have been found in LPI families. In addition, strong allelic association in the Finnish families implies that LPI in Finland is caused by a founder mutation.^{8,9}

Thus as in GGM, the discovery of altered genes coding for amino acid transporters provides a genetic explanation for two genetic diseases characterised by functional impairment of these transporters in the proximal tubule and small intestine. In addition, it provides a genetic explanation for the very different phenotypes and further strengthens the previous pathophysiological findings: cystinuria is a disease of the luminal membrane of the epithelial cells while LPI is a disease of the basolateral membrane.^{10,11} To the group of successful stories, where clinical history, cellular abnormalities, and targeted gene fit together, it is possible to add congenital malabsorption of bile salts¹² and Menke's disease, which is due to copper malabsorption.^{13,14}

In congenital transport diseases, as in many other areas of medicine, the discovery of a mutated gene may not entirely fit with or explain the phenotype. In cystic fibrosis, discovery of CFTR (cystic fibrosis transmembrane conductance regulator) has been a major advance for the geneticists and membrane physiologists because it merged the long parallel routes of the biochemists and electrophysiologists searching for the mechanism of electrogenic chloride secretion. It has also helped in the understanding of the genetic transmission of the disease. Concerning the clinical consequences, the existence of CFTR has not fully unravelled the pathophysiology of the disease or improved its treatment. One of the main limitations of the discovery is that animals expressing CFTR do not display the main symptoms of the disease, namely respiratory disease. However, at the intestinal level, they lack the cAMP dependent electrogenic chloride secretion which is a hallmark of the disease.¹⁵

Congenital chloride diarrhoea (CLD) is a consequence of a selective defect in intestinal Cl⁻ transport. It is an autosomal recessive disease characterised by lifelong secretory diarrhoea, with high faecal Cl⁻ concentration, starting in utero. CLD is caused by a mutation in the gene first known as DRA (down regulated in adenoma). The gene encodes a transmembrane protein belonging to the sulphate transporter family that may participate in the Cl⁻/HCO₃⁻ exchange in the ileum and colon. It is located on chromosome 7 close to the CFTR gene.^{16,17} Thus in this case, discovery of a mutated gene provided more information on the genetic mechanism than on the pathophysiology and therapeutic consequences of the disease.

Consequences for gastroenterology and internal medicine

The first example stems from the genetic input in GGM. We may now ask if there are any genetic disorders involving other sugar transporters.

Five functional mammalian facilitated hexose carriers (GLUT) and two glucose-Na⁺ cotransporters (SGLT) have been characterised by molecular or expression cloning.^{2,18} They all transport glucose but GLUT-5 is primarily a fructose carrier. GLUT-2 is found in tissues carrying a large glucose flux, such as the intestine, kidney, and liver. Their respective roles in the epithelial cell of the small intestine and kidney proximal tubule have been recently reviewed.

Isolated fructose malabsorption (IFM) is a clinical entity characterised by digestive symptoms after a fructose load. Its prevalence increases with increasing use of fructose as sweetener in processed food in the form of high fructose corn syrup. Mutations for the GLUT-5 gene were screened in a group of eight patients with IFM. The conclusion of the study was that IFM does not result from expression of mutant GLUT-5 protein¹⁹ and thus IFM awaits further explanation.

Nucleotide substitutions in GLUT-2, which belongs to the solute carrier family member 2 (gene symbol SLC2A2), are associated with susceptibility to non-insulin dependent diabetes mellitus. They are also associated with Fanconi-Bickel syndrome in patients. The syndrome comprises hepatic glycogenosis with glycosuria and aminoaciduria. Diarrhoea is a common symptom; it may be related both to deficient glut-2 protein and accumulation of glucose in the enterocyte. Three homozygous mutations in the GLUT-2 gene have been reported.²⁰

The different phenotypes under the general term of renal glycosuria may involve genetic alteration of five different monosaccharide transporters in the kidney. There are no digestive symptoms in this syndrome.⁶

Nephrology may provide another approach as there are many similarities between the kidney tubule and small intestine at the epithelial level. Primary hypomagnesaemia with secondary hypocalcaemia (PHSH, HSH, HOMG, or HMGX) is one of the congenital diseases presenting with hypomagnesaemic seizure that may result from a selective defect in intestinal magnesium absorption. A congenital aetiology is suggested by the onset of illness in early infancy. Clinical descriptions suggest genetic and pathophysiological heterogeneity. The intestinal defect is not well understood at present. In contrast, two interesting genetic spots have been identified in hypomagnesaemia of renal origin: one in Gitelman syndrome at SLC12A3 (also known as TSC or NCCT) that codes for the NaCl thiazide sensitive cotransporter²¹ and another in renal hypomagnesaemia with hypercalciuria and nephrocalcinosis at the paracellin-1 (PCLN-1) gene. PCLN-1 is located at the tight junction of the thick ascending limb of Henle and is related to the claudin family of tight junction proteins. It may control selective magnesium permeability through the tight junction.^{22,23} However, PCLN-1 is not expressed significantly in the intestine.

Thus this example indicates that magnesium, which is one of the most common minerals in the body, is handled in the tubule epithelium by several proteins, one being in the tight junction. Although it has not yet been found in the intestine, we can anticipate that selective loss of nutrients, minerals, or vitamins may be a consequence of genetic alteration of the proteins controlling the function of the tight junction.

What are the consequences of a genetic alteration that increases rather than decreases a selective transport system?

Wilson's disease and Menkes' disease are inherited disorders of copper metabolism. In the former, copper is in excess while in the latter it is lacking. The genes that mutate to give rise to these disorders encode highly homologous copper transporting ATPases.

Congenital sodium diarrhoea is a consequence of altered sodium-hydrogen exchange at the luminal membrane of the enterocyte. In Liddle's syndrome, familial hypertension is related to an increase in sodium handling by the body. It has been linked to one or several mutations of a gene coding for the epithelial Na⁺ channel (ENaC) controlling the rate limiting step in the process of transepithelial Na⁺ reabsorption in the distal nephron, distal colon, and airways.^{24 25}

In addition to genetically determined intestinal lipid transport defects, it is worth mentioning that familial combined hyperlipidaemia is probably the most prevalent genetically determined disorder of lipoproteins now recognised.³

Selective absorption of nutrients is under genetic control

Most nutrients can be selectively malabsorbed by the intestine.⁴ Most of the corresponding congenital diseases are monogenic. In a few diseases selectivity of the malabsorption has been linked to selectivity of the transporters; this is the case for GGM where the mutation for SGLT1 impairs absorption of the monosaccharides glucose and galactose but does not alter fructose absorption which is transported by a different protein (GLUT-2). With the development of appropriate methods to study the phenotype-genotype relationship, many other genes can be expected to be linked to specific nutrient transporters. Thus diseases will be better defined and treated. In addition, knowledge of the genetic control of nutrient absorption may have two nutritional consequences: firstly, knowledge of the regulation of gene expression will have short and long term consequences. In the short term, the many mediators present in the intestinal mucosa may directly or indirectly participate in the control of gene expression.²⁶ In addition, in paediatrics, we are very much concerned by the possible long term consequences of the type of feeding during pregnancy and early infancy. So far it has been difficult to study at the intestinal level. With the development of cDNA arrays that allow study of mRNA expression in a specific tissue, it might be possible to address these questions in the near future.²⁷

The second consequence is knowledge of the variability of intestinal transport capacity among individuals in a population. Deleterious mutation of a gene resulting in selective nutrient malabsorption can be considered as an extreme consequence of gene polymorphism (when searching in Medline/OMIM database, "deleterious" must be added to "polymorphism" to find genetic diseases). The frequent log normal distribution of an enzymatic activity or transport capacity may be considered as the functional

counterpart of the gene polymorphism. Thus for the future nutritional recommendations in a specific population it may be possible to include data on the polymorphism of genes coding for nutrient absorption.

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