Genetic influences on carbohydrate digestion

Dallas M. Swallow

*Galton Laboratory, Department of Biology, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK*

The diversity of the human genome leads to many functional differences between individuals. The present review focuses on genetic variations, both rare and common, that are of relevance to digestion of the sugars and starches that form a major part of human diets, and considers these in relation to the evolution of our species. For example, intolerances of dietary saccharides are not usually life-threatening because symptoms can be avoided by removal of the offending sugar from the diet, and deficiencies of the relevant enzymes are in some cases found at relatively high frequencies in certain populations. This is of evolutionary interest in relation to changes in the human diet, and the lactase-persistence polymorphism, in particular, provides an interesting model. More of the world’s adult population are lactase-deficient than have high lactase. The other deficiencies are however much more rare, but the significance of variant alleles at these loci, and also heterozygosity for deficiency alleles, to human nutrition and health is an area that is relatively unexplored.

Carbohydrate digestion: Lactase deficiency: Food intolerance: Human genetic variation

**Introduction**

Vertebrate species have an enormous diversity of diet and this is reflected in differing function at all levels from catching and eating of foods to metabolism of the digested products. It has long been known that in any species there is considerable inter-individual genetically determined variation. At the molecular level, we know that there are nucleotide differences between two unrelated individuals on average at intervals of less than 1 kb (Bentley, 2000). Some of these nucleotide differences are common variations or polymorphisms, and some are very rare. Although many of these differences are in non-coding and non-functional DNA, and many coding sequence changes are silent, polymorphism that affects function is not at all uncommon and can cause differences between individuals that may not be obvious unless the appropriate environmental circumstances are present. Probably at least one-third of all proteins have relatively common protein sequence variants (Harris, 1971), and others have variants in critical regulatory sequences.

As an omnivore, man has tremendous flexibility in diet, but although man can clearly survive on a broad range of diets, there are large inter-population differences, both in traditional and modern societies. Some, such as the Inuit, have an almost exclusively meat diet, while others had little access to meat. In some areas man has practised agriculture and animal husbandry for as long as 5000–10 000 years, while in others lived on only what could be collected or hunted. Even among agricultural societies, there is tremendous variation in the most important crops and the farming of animals.

Variation in diet is likely to have been a critical evolutionary pressure in the history of our species and a variety of anecdotal examples support an important role for diet in shaping patterns of human genetic variation. For example, there is a strong correlation between the lactose-tolerance phenotype and milk drinking, and also the geographic distribution of alleles of the lactase gene (Swallow & Hollox, 2000). Less is known about the significance of other variations in carbohydrate digestion. The present review considers molecular, genetic, and nutritional aspects of these variations, giving lactase, about which most is known, as a detailed example.

For references to gene symbols and gene mapping information, see [http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl](http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl) which also gives links to Online Mendelian Inheritance in Man (OMIM; [http://www.ncbi.nlm.nih.gov/Omim/](http://www.ncbi.nlm.nih.gov/Omim/)) and other literature and sequence links. The OMIM site provides a continuously updated source of references that can be found under the individual disease or gene names.
Dietary carbohydrates and their digestion

Digestion of starches commences with salivary and pancreatic amylases (EC 3.2.1.1) that are endoamylases and produce maltose (glucose α 1–4-glucose) and isomaltose (glucose α 1–6-glucose), oligosaccharides and some larger oligomers. The final hydrolysis of these occurs in the small intestine where the brush-border membrane maltase–glycoamylase and sucrase–isomaltase convert them to glucose (Semenza et al. 2000), which is taken into the absorptive cells by the Na+-dependent glucose transporter (SGLT) 1. Sucrase–isomaltase digests all the sucrose and 80% of the dietary maltose, while maltase–glucoamylase digests the remaining maltose and alone digests the glucose oligomers.

Dietary disaccharides include lactose, the main carbohydrate in human and other mammalian milks, sucrose from sugar beet and sugar cane, and a rather more rare dietary component, trehalose (glucose α 1–1-glucose), which is present in mushrooms, insects and certain seaweeds. These are hydrolysed by lactase, sucrase and trehalase (Semenza et al. 2000), respectively, before transport of the component monosaccharides by the brush-border sugar transporters. The bulk of the molecule of each of the hydrolases projects into the lumen of the intestine although the mode of anchorage into the membrane differs. The transporters, in contrast, span the membrane.

Dietary monosaccharides are present principally in fruits and are transported directly by the brush-border membrane transporters. Fructose, which is also one of the products of sucrose digestion, is transported by glucose transporter (GLUT) 5 while glucose and galactose are transported by SGLT1 (Levin, 1994; Wright et al. 2000).

There are thus several molecules involved in the digestion and uptake of carbohydrate that are of critical importance to normal carbohydrate digestion (Table 1). Many of the genes encoding these proteins have been shown to be genetically polymorphic or show rare deficiency variants.

Enzymes and their variation

Salivary and pancreatic amylases

The amylases present in saliva and pancreatic fluid are encoded by distinct closely linked genes, located on chromosome 1p21 (Groot et al. 1989, 1991). Polymorphism of both has been demonstrated in man.

The gene family consists of two pancreatic genes (AMY2A and 2B) (Yokouchi et al. 1990) and at least one salivary amylase gene (AMY1) (Nishide et al. 1986), the number of salivary amylase genes differing in different individuals. The short haplotype contains two pancreatic amylase genes and one salivary amylase gene (AMY1C) arranged in the order 2B–2A–1C. Other alleles contain extra gene copies and have a general structure: 2B–2A–(1A–1B–P1)n–1C where n can range from 0 (as in the short haplotype) to three copies of the repeated section and P1 is a pseudogene. Importantly, gene copy number polymorphism of the salivary amylase has been associated with variation in the level of enzyme activity (Groot et al. 1991), but to date this work has not been advanced further. The genetic differences between individuals do however indicate that as well as behavioural differences, such as the amount of mastication, there may be inherited differences in the extent of digestion of carbohydrate before it gets to the small intestine.

Intestinal hydrorases and transporters

Further variation (both rare and common) can be seen of the molecules at the apical surface of the small-intestinal absorptive cells. Reduced function of any one of these leads to the passage of undigested carbohydrate into the bowel where it is fermented by bacterial flora to form short-chain fatty acids and gases. While fatty acid absorption increased by colonic fermentation is thought by some to be beneficial, excessive production of gases leads to flatulence and the osmotic effect of di- and monosaccharides in the luminal contents leads to diarrhoea. These symptoms can be life-threatening when accompanied by other disease, starvation or inappropriate food aid, providing a very strong negative selective force against deficiency in the context of diets that include those substrates.

Congenital milk intolerances

The presence of both lactase and SGLT1 is essential for suckling mammals. Deficiency of either is very serious, and can be fatal if not diagnosed immediately, since there is onset of symptoms (diarrhoea and failure to thrive) as soon as milk is first consumed.

Congenital glucose and galactose malabsorption

Congenital glucose and galactose malabsorption is due to deficiency of SGLT1 (Wright et al. 2000). SGLT1 is encoded by the 80 kb gene SLC5A1 located on chromosome 22. It is a glycoprotein of relative molecular mass of approximately 73 000 with fourteen membrane-spanning domains and is the major apical transporter of glucose and galactose. Many different mutations have been found throughout the length of the molecule and each of these is rare so that patients are often compound heterozygotes. Most of the mutations result in targeting defects or incomplete synthesis of the protein. A special diet is required in which glucose, galactose and lactose are eliminated. A carbohydrate-free formula supplemented with fructose can be used in early childhood but later an avoidance strategy has to be adopted, which means a diet rather rich in protein and fat.

Congenital alactasia and adult hypolactasia

Lactase (or lactase–phlorizin hydrolase) is encoded by the 60 kb gene LCT on chromosome 2q21. The enzyme has two activities (EC 3.2.1.23, 3.2.1.62), a β-galactosidase activity hydrolysing lactose, and a β-glucosidase activity (Wacker et al. 1992; Zecca et al. 1998; Arribas et al. 2000) capable of hydrolysing phlorizin, a disaccharide found in roots and bark of plants of the family Rosaceae and some seaweeds. The mature lactase–phlorizin hydrolase enzyme contains two related domains harbouring the two active sites (Arribas et al. 2000), but at least in rats it appears that...
both domains need to be present to allow lactase activity (Jost et al. 1997). Lactase–phlorizin hydrolase can also hydrolyse other plant glycosides such as flavonoid glycosides (Day et al. 2000) and pyridoxine-5’-β-D-glucoside (Armada et al. 2002; Mackey et al. 2002), apparently using the lactase active site.

**Congenital alactasia.** Congenital deficiency of lactase or alactasia is even more rare than congenital glucose and galactose malabsorption, though a cluster of cases has been reported in Finland (Savilahit et al. 1983) where this is one of the ‘Finnish recessive disorders’.

Very little is known about the molecular basis of this condition. No coding mutations have yet been reported, but the locus responsible for Finnish congenital alactasia has been genetically mapped 5'/H11032 of LCT (Jarvela et al. 1998) and these authors suggest that it is at a considerable distance from the gene (2 Mb).

**Lactase persistence and non-persistence.** While congenital lactase deficiency is rare, adult lactase deficiency, in contrast, is common. Lactase fails to persist into adult life in some individuals but not in others and this non-persistence is in fact globally more common than lactase persistence. This is a genetically determined polymorphism in human populations, which involves different developmental regulation and which shows large differences in allele frequency in human populations (Swallow & Hollox, 2000).

The expression of lactase mRNA is tightly controlled; it is expressed at low levels in fetal life and increases around birth and it is only expressed in small-intestinal enterocytes (Wang et al. 1998). Lactase expression declines some time after weaning in most mammals and many human individuals but persists into adult life in many others.

Lactase persistence tends to be the most frequent phenotype in populations where fresh milk forms a significant part of the adult diet, for example, Northern Europeans and pastoral nomadic tribes (Swallow & Hollox, 2000). Lactase-non-persistent adults can usually consume only limited amounts of fresh milk without experiencing flatulence and diarrhoea. The very high frequency of the lactase-persistence allele in certain populations probably results from a combination of natural selection and genetic drift. Selection for milk drinking may have been due to the nutritional value of milk, and in the case of Northern climes, with low sunlight, possibly because of its Ca content. The water content of milk may have conferred a selective advantage in the case of desert pastoralists (Swallow & Hollox, 2000). One group have put forward the hypothesis that selection against lactase persistence and late weaning, by malaria, might account for the near absence of this allele in much of Africa (Anderson & Vullo, 1994), but others have produced counter-evidence (Meloni et al. 1996, 1998, Auricchio, 1998).

The lactase persistence–non-persistence polymorphism is controlled by a cis-acting regulatory element (Wang et al. 1995, 1998; Enattah et al. 2002). Study of haplotypes comprising eleven polymorphisms distributed across the 70 kb lactase gene in different human populations showed that very few haplotypes occur in most populations, but that there is much greater haplotype diversity in African popula-
tions (Hollox et al. 2001). One particular haplotype, A, is associated with lactase persistence and is at very much higher frequency in Northern Europe than any other population, suggesting a selective sweep by the linked alleles responsible for the phenotypic polymorphism. We have characterised a hypervariable region immediately upstream from the lactate gene and shown evidence of variable binding to a possible trans-acting protein (Hollox et al. 1999) although this region does not correspond to the critical causative element.

A putative causative single nucleotide polymorphism has now recently been described (Ennatah et al. 2002), at 14 kb from the gene, in an intron of the adjacent gene, but at present it cannot be formally excluded that this single nucleotide polymorphism is not just a highly associated marker. The most obvious hypothesis is that genetic differences in this sequence element cause it to interact differentially with a developmentally regulated trans-acting protein(s). However our recent studies (Poultier et al. 2003) and those of Rossi et al. (1997) suggest that things are more complicated than this.

Lactose-intolerant individuals usually do not consume large volumes of fresh milk. The nutritional consequences of this have long been a matter of controversy. Fresh milk supplies an excellent source of dietary Ca, which is generally perceived to be beneficial, although some authors have suggested that it confers increased risk of heart disease (Seely, 2000). Likewise the higher consumption of galactose has been implicated in risk of cataract (Birlouez-Aragon et al. 1993). The potential risks or benefits of digestion of plant glycosides have not yet been considered.

Sucrase–isomaltase deficiency

The enzyme sucrase–isomaltase (EC 3.2.1.48, 3.2.1.10) is encoded by a 57 kb gene (SI) on human chromosome 3q. SI encodes an mRNA transcript of 5.48 kb and a 1827 amino-acid peptide precursor (Chantret et al. 1992). Sucrase–isomaltase is also a two active site molecule, synthesised as a single polypeptide chain (pro-sucrase–isomaltase), which is cleaved by a pancreatic protease to form the two sub-units with different substrate specificity and is anchored to the brush-border membrane by an N-terminal hydrophobic anchor (Semenza et al. 2000).

Sucrase–isomaltase deficiency is probably not uncommon in several different populations. Sucrose intolerance reaches frequencies as high as 10% in Inuit populations (McNair et al. 1972; Bell et al. 1973; Asp et al. 1975; Ellestad-Sayed et al. 1978; Meier et al. 1991; Gudmand-Hoyer & Skovbjerg, 1996), societies who traditionally ate an almost exclusively meat diet. Severity of the condition depends directly on the dietary sucrose intake and can be particularly harmful in young children. Sucrose had not formed part of the Inuit diet until it was introduced by the Danes. Sugar was popular and the connection between sugar intake and quite severe symptoms was not made until the 1970s. It has generally been assumed that a deficiency allele reached polymorphic frequencies due to genetic drift, in the absence of selection against it.

Deficiency of sucrase–isomaltase is, however, also found in about 2% diagnostic biopsies in London (AD Phillips, CB Harvey, P Clay, DM Swallow and JA Walker-Smith, personal communication) and in the USA (Ringrose et al. 1979) and there are reports of reduced sucrase–isomaltase in black South Africans which suggest the possibility of activity polymorphism (Veitch et al. 1998). Studies on the sucrase–isomaltase protein in individuals of European extraction suggest that various different mutations cause the deficiency, and so far three exonic mutations have been identified. One results in substitution of proline by a glutamine at amino acid 1098 (Ouwendijk et al. 1998) and one causes the protein to be secreted (Jacob et al. 2000), and one glutamine by arginine 117 affects apical protein transport (Spødsberg et al. 2001). It is not known whether any one mutation occurs at higher frequency.

A proportion of sucrase-deficient patients are not completely cured by removal of sucrose from the diet (Treem, 1996). This is probably because as well as digesting sucrose, this enzyme is responsible for most of the digestion of maltose.

Maltase–glucoamylase deficiency

Maltase–glucoamylase (EC 3.2.1.20) is encoded by a 63 kb gene, MGAM, located on chromosome 7. The mRNA transcript is 6513 bp and calculated relative molecular mass of the protein (before glycosylation) is 209 702 (Nichols et al. 1998). This protein probably forms homodimers and, like sucrase, the complex is anchored to the brush-border membrane by the N-terminus of the polypeptides. Analysis of the human maltase–glucoamylase sequence shows that the catalytic sites are duplicated and are identical to those in sucrase–isomaltase, but overall the protein sequence is only 59% identical. However the intron–exon structure of these two genes is highly conserved and it is quite clear that an ancestral maltase duplicated to give a double-function molecule before this itself duplicated and the two molecules diverged in function (Nichols et al. 2003). It is not entirely clear how early this was on an evolutionary timescale but sequence comparisons suggest duplication of the two genes 10^7–10^8 years before the present (Nichols et al. 2003). Both molecules are present in pigs and mice (Calvo et al. 2000; Quezada-Calvillo et al. 2002), but interestingly sheep lack sucrase activity (Shirazi-Beechey et al. 1989).

The only case of maltase–glucoamylase deficiency for which sequence analysis has been reported showed that an associated deficiency of sucrase–isomaltase and lactase and a defect in the MGAM gene was unlikely to be the cause of this child’s problem (Nichols et al. 2002). However, through study of this child we identified a polymorphism that causes an amino-acid change close to the active site of the protein. While transfected constructs carrying this nucleotide change show activity towards maltose it is intriguing to speculate that they may show altered kinetics and have functional significance. A large number of other single nucleotide polymorphisms have also been identified. It would be of interest to investigate polymorphism of this gene in relation to irritable bowel syndrome, and inter-personal differences in response to oligosaccharide sweeteners.
**Trehalase deficiency**

A human mRNA transcript of 2 kb long encodes a trehalase (**EC** 4.1.2.13) protein with a calculated molecular weight of 66 595 Da, which is expressed in the kidney and liver as well as the intestine (Ishihara et al. 1997). The 13 kb gene, **TREH**, has been mapped to human chromosome 11q23 (Oesterreicher et al. 2001). Experiments using in vitro transcribed rabbit trehalase mRNA injected into Xenopus laevis oocytes and treatment with phospholipase C have suggested that, unlike lactase, sucrase–isomaltase and maltase–glucoamylase, trehalase is attached to the brush-border membrane by a phosphatidylinositol anchor (Ruf et al. 1990).

Trehalase deficiency is thought to be an autosomal recessive trait and has been reported at a frequency of 8% in Greenland (McNair et al. 1972) but has also been reported in a parent and child and three other close relatives (Madzarovova-Nohejlova, 1973) suggesting dominant inheritance. Given the unusual distribution of the disaccharide, deficiency has not been of any nutritional consequence until recently, but may become a problem because of its introduction as a sweetener in foods. This will undoubtedly bring other cases to light.

**Glucose (fructose) transporter**

GLUT5 is an apical glucose transporter whose main role seems to be the transport of fructose (Levin, 1994). It also has many trans-membrane domains and it is encoded by the gene SLC2A5 located on chromosome 1p36.

Fructose malabsorption with the classical symptoms of diarrhoea and flatulence has been described and was thought to be likely to be due to a transporter defect, but sequencing revealed no mutations in the coding sequence of GLUT5 (SLC2A5) (Wasserman et al. 1996).

**Hereditary fructose intolerance**

Intolerance of dietary fructose can have a quite different clinical presentation from that described above. A somewhat more common and potentially severe condition manifests as recurrent hypoglycaemia and vomiting, failure to thrive, and kidney and liver involvement. In this condition deficiency of fructose phosphate aldolase (aldolase B) has been found. Aldolase (**EC** 4.1.2.13) catalyses the conversion of fructose-1-phosphate to dihydroxyacetone phosphate and D-glyceraldehyde, and is thus essential for the metabolism of dietary fructose, which is transported to the liver from the enterocytes via the basolateral GLUT2. Aldolase B is encoded by a gene **ALDOB** located on chromosome 9q21/22.

A number of different mutations leading to amino acid substitutions have been found in **ALDOB**, but a single mutation is found at a heterozygosity of more than 0.01 in the UK, for example (Ali et al. 1998). The high incidence of the deficiency alleles suggests that before the recent increase in intake of dietary fructose there was little selective disadvantage for this allele. The condition varies in its clinical severity according to the dietary intake of fructose and removal of fructose from the diet prevents the disease. It is of interest to note that in patients who have consciously or unconsciously avoided fructose (and often escaped diagnosis) there is a remarkable absence of dental caries.

**Discussion**

The present article reviews examples of the inter-relationship between genetic variation and carbohydrate components of the human diet. Although several of the examples are of very rare ‘inborn errors of metabolism’, in some cases the heterozygote frequency may not be so very low. It is, for example, uncertain how frequently genetic deficiencies of sucrase–isomaltase, trehalase and maltase–glucoamylase occur in different human populations, but it is probable that in some cases the allele frequency is sufficiently high to give a reasonable heterozygote frequency. Before the advent of high-starch diets, there may have been little selection against loss of one of the β-glycosidase activities in early man, because of their overlapping substrate specificity. However, their deficiency will be of much greater significance in modern diets.

Because enzymes are catalysts, half levels of enzyme activity, as usually found in heterozygotes, are in most cases more than enough to be compatible with normal function. However, the complexities of sub-unit and protein–protein interactions as well as physiological changes in the individual due to ill health, dietary deficiencies, drug treatment and ageing may mean that heterozygotes are functionally compromised. Furthermore, in the context of the human intestine the anchorage of these molecules in the enterocyte membrane means that they are not free to diffuse. It is thus of interest that there are indications from animal experiments (Weiss et al. 1998) that the small-intestinal hydrolases and transporters, which are membrane-bound, are only present in marginal excess over the dietary load.

It is possible, if not probable, that there are many other more common variant alleles for proteins of the intestinal epithelium that alter the uptake of macro- and micronutrients. Many of the dietary micronutrients, which have to be absorbed through the intestine via active transport mechanisms, are present in rather low (limiting) amounts in some diets. The advent of cereals as the staple source of carbohydrate means that, in comparison with the foods of pre-agricultural man, many diets are much higher in plant starches and can be relatively deficient in specific micronutrients. It is thus easy to imagine that variants of micronutrient transporters with only a modestly altered effect could be of considerable significance to nutrition. Some such variants may, like lactase, have been selected for in relatively recent human population history.

In conclusion, the high level of inter-individual variability means that dietary requirements may well differ from individual to individual. Although the majority of claimed food sensitivities may be due to influences of the media and inappropriately controlled subjective observations, there is no doubt that conditions such as irritable bowel syndrome may in part result from specific food intolerances for which there is a genetic basis.
References


Genetic influences on carbohydrate digestion


