Human CAZyme genes polymorphism and risk of IBS: a population-based study

A series of papers in *Gut* recently highlighted genetic variation in the sucrase-isomaltase gene (*SI*; coding for a brush-border disaccharidase) as a likely causative factor in a subset of patients with irritable bowel syndrome (IBS).[1–4] Hypomorphic (dysfunctional) *SI* variants may thus underlie gastrointestinal symptoms in rare recessive forms of congenital SI deficiency (CSID)[5] as well as milder complex (IBS) manifestations,[6] across a broad spectrum of *genetic SI deficiencies* (GSID) that vary in severity and onset of presentation.[7] Moreover, *SI* carrier status has been shown to also affect the response to specific carbohydrate-focused diets, thus providing a rationale for personalising (dietary) therapeutic strategies in IBS.[1 8]

Together with SI, a number of human Carbohydrate-Active enZymes (hCAZymes, www.cazy.org/e355.html) are involved in the breakdown of polysaccharides during the process of carbohydrate digestion, which is initiated by salivary amylases (AMYs) and finalised in the small intestine by pancreatic AMYs and brush-border disaccharidases SI, lactase (LCT), maltase-glucoamylase (MGAM) and trehalase (TREH) (figure 1).[5] Of note, similar to *SI* in GSIDs, mutations in other hCAZymes cause rare genetic forms of carbohydrate maldigestion, while regulatory DNA variations (persistent genotype) influence lactose intolerance in adults.[5] This suggests hCAZyme genes other than *SI* may contribute to IBS predisposition via similar mechanisms (reduced hCAZyme activity increasing IBS risk): we sought to test this hypothesis through the analysis of genetic and health-related data in 366,432 individuals of European ancestry from the large population-based cohort UK Biobank (UKBB) detailed methods' description is provided in online supplemental material).

Patients with IBS were identified across four definitions, based on alternative diagnoses from hospital admissions, general practitioner's notes, Digestive Health Questionnaires including Rome III Criteria and as self-reported condition from health-related questionnaires (online supplemental table S1). Rare (allele frequency<1%) hCAZyme functional (missense, nonsense, read-through and splice-site) DNA variants were extracted from UKBB whole-exome sequencing data for nine genes of interest (*AMY1A*, *AMY1B*, *AMY1C*, *AMY2A*, *AMY2B*, *LCT*, *MGAM*, *SI* and *TREH*), and hypomorphic variants computationally predicted using stringent criteria from Ensembl Variant Effect Predictor and the pathogenicity classifier AlphaMissense. UKBB participants were stratified into carrier and non-carrier groups for each gene, and hCAZyme-IBS associations were tested via adjusted logistic regression. Additionally, cumulative analyses were carried out for selected genes, in which carrier status was considered collapsing hypomorphic variants from multiple genes into a single group.

In total, 1714 hypomorphic variants were identified across hCAZyme genes (online supplemental table S2). As reported in table 1, when individually tested for association with IBS (based on different definitions), three hCAZyme genes showed significant effects on disease risk after correction for multiple comparisons, namely *SI* (as previously shown),

Table 1 Significant associations between hCAZymes genes and risk of IBS

	IBS definition	OR (95% CI)	P value	P_{FDR}
Single-gene analyses*				
SI	*Self-IBS*	1.1 (1.0 to 1.3)	0.0101	0.0296
SI	*HA-IBS*	1.1 (1.0 to 1.3)	0.0203	0.0296
SI	*Rome-IBS*	1.1 (1.0 to 1.2)	0.0222	0.0296
AMY1B	*HA-IBS*	1.7 (1.1 to 2.7)	0.0078	0.0311
AMY2A	*HA-IBS*	1.5 (1.1 to 2.2)	0.0079	0.0317
Multiple gene analyses†				
Any gene	*HA-IBS*	1.2 (1.1 to 1.3)	0.0015	0.0059
Any gene	*Self-IBS*	1.2 (1.0 to 1.3)	0.0030	0.0061
Any gene	*Rome-IBS*	1.1 (1.0 to 1.2)	0.0143	0.0190
Number of genes affected	*HA-IBS*	1.2 (1.1 to 1.3)	0.0011	0.0045
Number of genes affected	*Self-IBS*	1.2 (1.1 to 1.3)	0.0040	0.0079
Number of genes affected	*Rome-IBS*	1.1 (1.0 to 1.2)	0.0151	0.0201

*Studying carriers of hCAZyme hypomorphic variants in individual genes.
†Studying carriers of hCAZyme hypomorphic variants in any gene (*AMY1B*, *AMY2A* or *SI*) and as a function of the number of genes affected.
AMY, amylase genes; HA-IBS, Hospital inpatient Admissions IBS; hCAZymes, human Carbohydrate-Active enZymes; IBS, irritable bowel syndrome; Rome-IBS, Rome III Criteria from the Digestive Health Questionnaire IBS ; Self-IBS, Self-reported IBS; *SI*, sucrase-isomaltase gene.

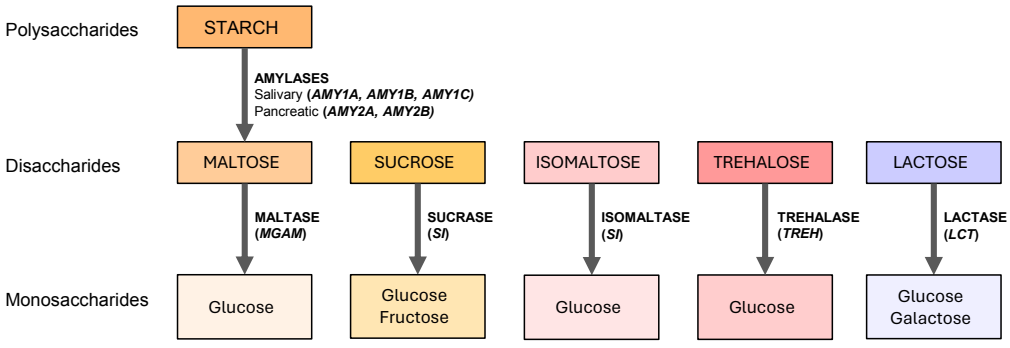

Figure 1 Schematic representation of the process of carbohydrate digestion. Reported are the types of carbohydrates, the hCAZymes involved and the corresponding genes studied here.

AMY1B (a salivary AMY) and *AMY2A* (a pancreatic AMY). Carriers of a hypomorphic variant in any of these genes were also exposed to increased risk of IBS (table 1), while the strongest association was detected in relation to the number of genes affected by hypomorphic variation (table 1). No other significant associations were detected (not shown).

These results confirm and extend previous findings on the importance of hCAZyme genotype in relation to IBS risk.[6 7] *AMY1B* and *AMY2A* code for AMYs that break down starch into smaller sugars and disaccharides, hence their reduced activity may ultimately lead to excess carbohydrates in the lower bowel where they induce IBS symptoms via osmotic diarrhoea and bacterial fermentation. Of note, *AMY1* and *AMY2* copy numbers (a type of genetic variation not studied here) have been shown to affect AMY activity and starch intake, as well as oral and gut microbiota composition.[9 10]

In summary, we provide additional evidence that hCAZyme genotype is relevant to IBS risk. This warrants replication of current findings, as it holds potential implications for personalising (dietary) therapeutic approaches in IBS.

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