Functional Variation in Human CAZyme Genes in Relation to the Efficacy of a Carbohydrate-Restricted Diet in IBS Patients

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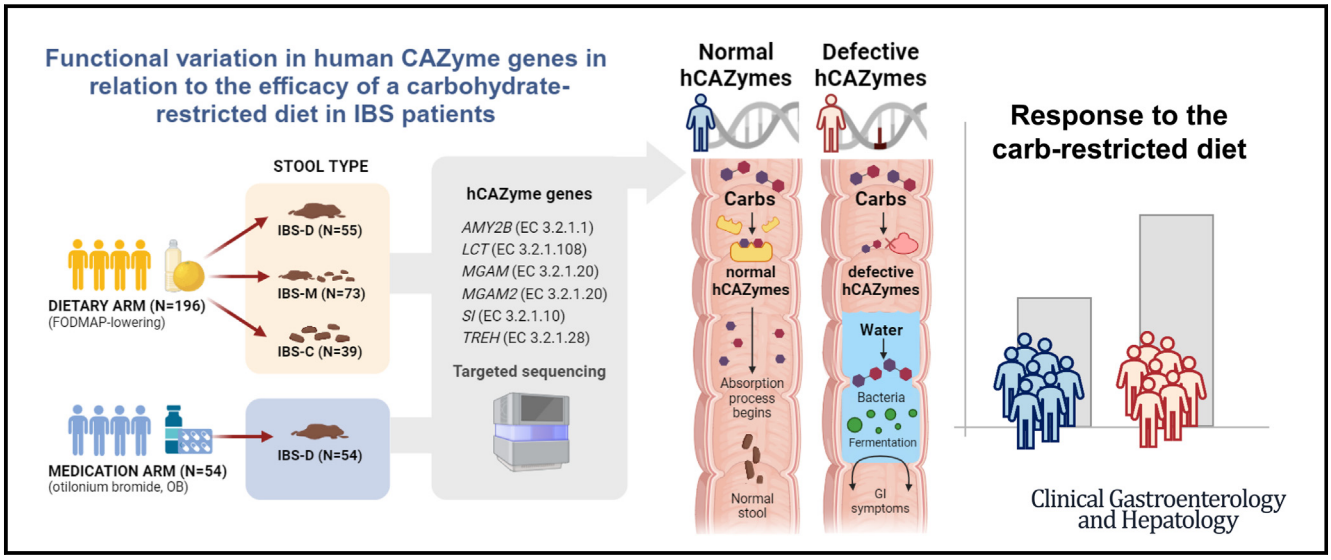

BACKGROUND & AIMS: **Limiting the dietary intake of certain carbohydrates has therapeutic effects in some but not all irritable bowel syndrome (IBS) patients. We investigated genetic variation in human Carbohydrate-Active enZYmes (hCAZymes) genes in relationship to the response to a FODMAP-lowering diet in the DOMINO study.**

METHODS: **hCAZy polymorphism was studied in patients with IBS from the dietary (FODMAP-lowering; n = 196) and medication (otilonium bromide; n = 54) arms of the DOMINO trial via targeted sequencing of 6 genes of interest (*AMY2B*, *LCT*, *MGAM*, *MGAM2*, *SI*, and *TREH*). hCAZyme defective (hypomorphic) variants were identified via computational annotation using clinical pathogenicity classifiers. Age- and sex-adjusted logistic regression was used to test hCAZyme**

Abbreviations used in this paper: FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; hCAZymes, human carbohydrate-active enzymes; IBS, irritable bowel syndrome; IBS-D, IBS with diarrhea; IBS-SSS, IBS-Symptom Severity Score; OB, otilonium bromide.

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polymorphisms in cumulative analyses where IBS patients were stratified into carrier and non-carrier groups (collapsing all hCAZyme hypomorphic variants into a single bin). Quantitative analysis of hCAZyme variation was also performed, in which the number of hCAZyme genes affected by a hypomorphic variant was taken into account.

RESULTS: **In the dietary arm, the number of hypomorphic hCAZyme genes positively correlated with treatment response rate ($P = .03$; odds ratio = 1.51; confidence interval = 0.99–2.32). In the IBS-D group (n = 55), hCAZyme carriers were 6 times more likely to respond to the diet than non-carriers ($P = .002$; odds ratio = 6.33; confidence interval = 1.83–24.77). These trends were not observed in the medication arm.**

CONCLUSIONS: **hCAZYme genetic variation may be relevant to the efficacy of a carbohydrate-lowering diet. This warrants additional testing and replication of findings, including mechanistic investigations of this phenomenon.**

Keywords: Irritable Bowel Syndrome; Functional Polymorphism; Nutrigenetics; lowFODMAP Diet; Carbohydrate Maldigestion; CAZyme.

The pathophysiology of irritable bowel syndrome (IBS) is multifactorial, contributed to by several risk factors: psychological stressors, gut dysbiosis, epithelial barrier dysfunction, mucosal immunity, and dietary triggers.[1–4] The latter in particular might explain some of the clinical manifestations associated with postprandial IBS symptoms,[5] and avoidance of carbohydrates (owing to their potential maldigestion) is recommended as a first-line approach by the UK England and Wales National Institute for Health and Care Excellence guidelines for IBS. Limiting the dietary consumption of certain carbohydrates, especially those poorly absorbed in the small intestine and known as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs), also seems to have therapeutic effects in some but not all IBS patients.[6–11] The food-symptom relationship in IBS may involve maldigestion of carbohydrates due to inefficient enzymatic breakdown of polysaccharides, which can be fermented by colonic microbiota generating gas and short-chain fatty acids ultimately affecting gastrointestinal function.[12]

Polysaccharide breakdown is a process that, in humans, involves the action of several human Carbohydrate-Active enZYmes (hCAZymes; http://www.cazy.org/e355.html) (Figure 1). Carbohydrate digestion is initiated by salivary and pancreatic amylases (AMYs) and then finalized in the small intestine by other glycosidases (brush-border enzymes, such as lactase - LCT, sucrase-isomaltase - SI, maltase-glucoamylase - MGAM, and trehalase - TREH) that hydrolyze disaccharides into monosaccharides ultimately absorbed by the enterocytes. Highlighting this, hCAZyme mutations cause rare genetic forms of carbohydrate intolerance, whereas regulatory DNA variations (persistence genotype) influence lactose intolerance in adults.[13]

While genetic forms of carbohydrate maldigestion present with clinical manifestations overlapping with (or misdiagnosed as) IBS,[14] compelling evidence for similar mechanisms underlying bowel symptoms in a subset of IBS patients is accumulating, mostly in relationship to the *SI* gene: in a series of case-control and population-based studies *SI* hypomorphic variants with (experimentally verified and/or computationally predicted) reduced enzymatic activity have been shown to confer an increased risk of IBS (reviewed in Ref [15]) and to affect the response to lowFODMAP and sucrose- and starch-reduced diets.[16,17] This suggests similar mechanisms may be detected for other hCAZymes involved in the breakdown of carbohydrates found in foods that are typically restricted in carbohydrates-focused diets. We sought to test this hypothesis in a pilot analysis of the correlation between hCAZyme gene carrier status and response to a carbohydrate-restricted (FODMAPs-lowering) intervention from the Diet Or Medication in Irritable Bowel syNdrOme (DOMINO) trial. The outcome of this type of study may have implications in the management of IBS, by means of allowing the design of new approaches for personalizing dietary intervention in a subset of genetically exposed IBS patients.

Materials and Methods

Patients Cohort

The current study is based on a retrospective (genetic) analysis of patients with IBS from the DOMINO trial (NCT04270487), previously described.[18] Briefly, Belgian IBS patients (N = 459) were randomized to either a dietary treatment (FODMAPs-lowering; dietary arm; n = 218) or administration of an antispasmodic agent (otilonium bromide [OB]; medication arm; n = 217), for 8 weeks.[18] Based on data from IBS Symptom Severity Score (IBS-SSS) questionnaires, a positive response was defined as a drop of 50 IBS-SSS points, compared to baseline. For the purpose of hCAZyme genetic analyses, 196 IBS patients from the dietary arm (39 IBS with constipation [IBS-C], 55 IBS with diarrhea [IBS-D], 73 IBS with

mixed type [IBS-M], and 29 IBS unclassified [IBS-U]), and 54 IBS-D patients from the medication arm were included in this study. The demographics, clinical characteristics, stool type, IBS-SSS profiles, and response to treatment of these patients are reported in Supplementary Table 1.[18] The study protocol was approved by the Ethical Committee Research UZ/KU Leuven (protocol S59482), and all participants provided informed consent.

Identification of hCAZyme Hypomorphic Variants

A detailed description of the methodology adopted to select and characterize hCAZyme genes, including their targeted next-generation sequencing and the identification of hypomorphic variants, is reported in the Supplementary Methods.

Statistical Analyses

Statistical analyses were performed with R (version 4.2.1)[19] in RStudio (v2022.07.2+576). R packages ggplot2[20] and dplyr[21] were used for data visualization. hCAZyme hypomorphic variants were tested versus the response to treatment in cumulative analyses, collapsed into a single hypomorphic hCAZyme group. Accordingly, DOMINO participants were defined as carriers or non-carriers depending on whether they had at least 1 hypomorphic variant in 1 or more of the hCAZyme genes (carriers), or no hypomorphic variant (non-carriers), while the number of hCAZyme genes affected by hypomorphic variants was also taken into account in separate analyses (Figure 2). hCAZyme carrier status (independent variable, binary) and number of hCAZymes affected genes (independent variable, continuous) were tested for their effect on treatment response (dependent variable, binary) using logistic regression (1-tail) adjusting for participants' sex and age. Similar analyses were carried out for IBS subtypes. Fisher exact test was used to test *SI* and hCAZyme variants in sensitivity analyses because of small sample sizes and the presence of 0 values in the outcome variable groups.

What You Need to Know

Background

Carbohydrates are known triggers of IBS symptoms, and limiting their dietary consumption appears to have therapeutic effects

Carbohydrate-restricted diets improve symptoms in some but not all IBS patients

Findings

Carrying hypomorphic hCAZyme gene variants associates with increased efficacy of a FODMAP-lowering diet in IBS, especially in patients with diarrhea (IBS-D)

Implications for patient care

hCAZyme genotype information may be relevant to increase therapeutic precision in IBS, contributing to personalizing carbohydrate-focused dietary interventions

Results

Identification of Human Carbohydrate-Active Enzymes Hypomorphic Variants

After sample and next-generation sequencing quality controls (Supplementary Methods), a total of 196

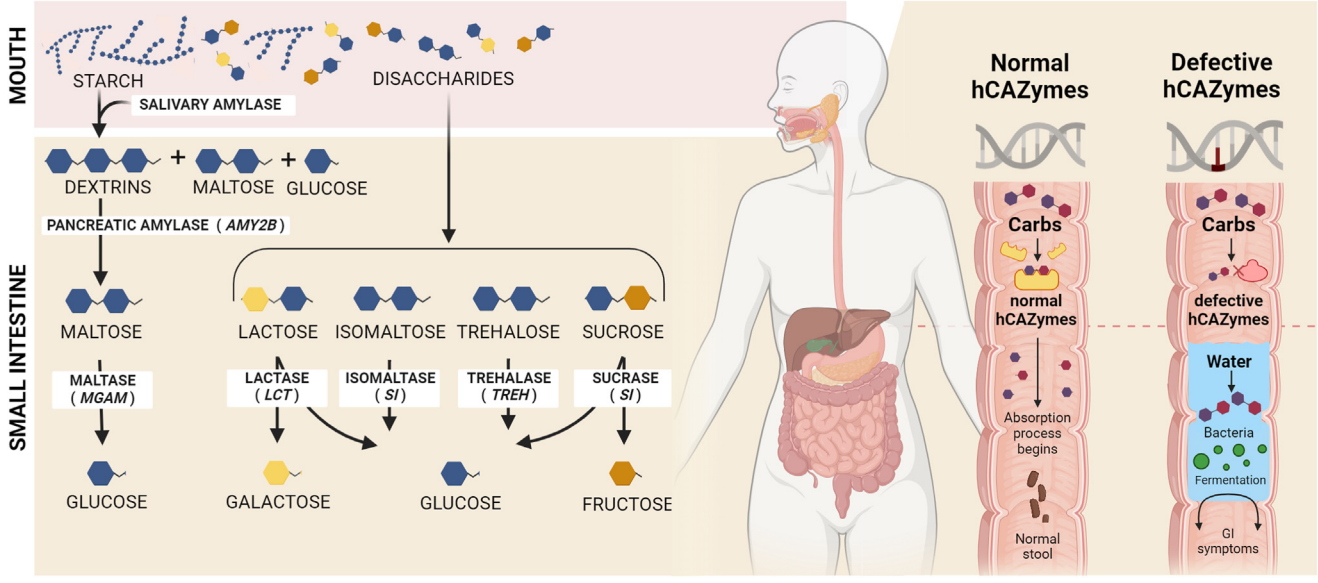

Figure 1. Graphical representation of the process of carbohydrate digestion in the gastrointestinal system, including defects in hCAZymes.

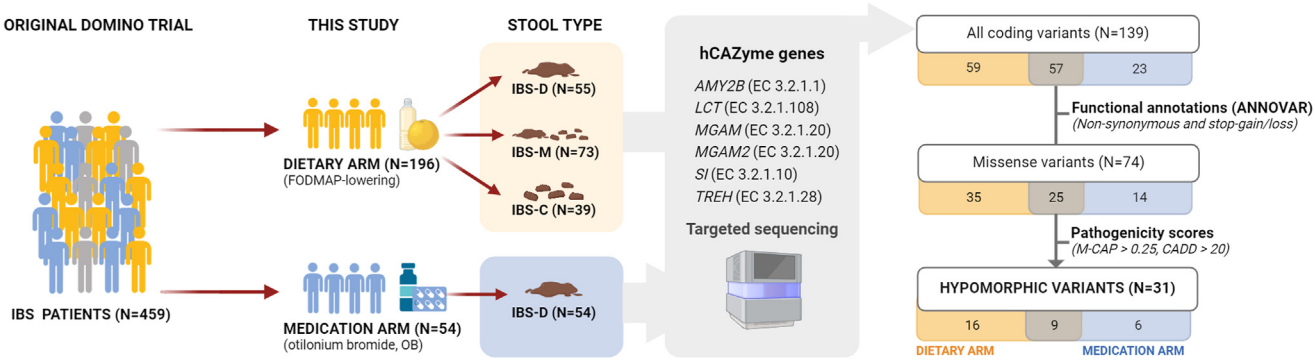

Figure 2. Graphical representation of the methodology used in this study (flowchart).

DOMINO patients from the dietary arm were deemed eligible for inclusion in the genetic analyses. In these individuals, we detected a total of 60 missense hCAZyme variants, all previously identified in other populations (present in the gnomAD genome reference database) (Figure 2).[22] These included 49 rare and 11 common hCAZyme variants (allele frequencies respectively below or above 5% in gnomAD), which are reported in Supplementary Table 2. Based on computational predictions of the functional effects of these amino acid changes (see Supplementary Methods), 25 hCAZyme variants were classified as hypomorphic (dysfunctional), the remaining 35 as benign. Most hypomorphic variants were rare and only found in single individuals, whereas 2 were common and detected in 18 (Leu1174Met, rs73547325 single-nucleotide polymorphism in *MGAM2*) and 113 (Val15Phe, rs9290264 single-nucleotide polymorphism in *SI*) patients, respectively (Table 1, Supplementary Figure 1). Multiple hypomorphic variants from different hCAZyme genes co-occurred in single individuals (Supplementary Figure 1).

hCAZyme Genes and Response to Dietary Treatment

DOMINO IBS patients treated with the FODMAP-lowering diet were stratified in hCAZyme carrier and non-carrier groups. While their demographic and clinical characteristics were similar (Supplementary Table 3), hCAZyme carriers generally exhibited more severe symptoms at baseline (Supplementary Figure 2), especially in the IBS-D group where significant associations were detected both for total IBS-SSS and individual symptoms scores (higher in single and multiple hCAZy carriers compared with non-carriers) (Figure 3). Responders to the diet generally showed increased prevalence of hypomorphic variants in individual hCAZyme genes (Supplementary Figure 3). When the outcome of the dietary treatment was studied in a sex- and age-adjusted logistic regression (see Methods section), a significant association between hypomorphic hCAZyme gene number and response rate was detected: the diet was more effective in patients with multiple (computationally predicted) defective hCAZyme genes ($P = .03$; OR = 1.51), reaching a 100% success rate in the small group of IBS patients carrying defective variants in 3 hCAZyme genes (Figure 4). A similar pattern was also observed at the level of severity of individual symptoms (from IBS-SSS), which tended to correlate with the number of affected hCAZyme genes (Supplementary Figure 4). Near-significant findings were also obtained for carriers of hypomorphic hCAZyme genes who were more likely to respond to the dietary treatment ($P = .06$; OR = 1.69) (Supplementary Figure 5). As shown in Figure 5*A*, this phenomenon was most pronounced in the IBS-D subtype, with hCAZyme carriers >6 times more likely to respond to the FODMAP-reducing diet than non-carriers as assessed on the IBS-SSS severity scale both at the global ($P = .002$; OR = 6.33) and, as a tendency, individual symptom level (Supplementary Figure 6). No significant findings were detected for the IBS-M and IBS-C subtypes (not shown).

SI *Sensitivity Analyses*

SI hypomorphic variants (particularly the common Val15Phe variant) were the most common hCAZyme variants in DOMINO and have been previously shown to negatively affect the response to a lowFODMAP diet,[16] hence we ran additional sensitivity analyses. There was no significant association with the response to dietary treatment when the whole DOMINO cohort was stratified into *SI* hypomorphic carriers and non-carriers (irrespective of other hCAZyme variants) and, similar to most other hCAZyme genes, *SI* hypomorphic variants were more common in responders, particularly those from the IBS-D group ($P = .044$; Supplementary Figure 3). Excluding *SI* carriers from the analyses confirmed near-significant higher response rate in hCAZyme carriers despite sample size reduction (N = 81; 18/22 = 81.8% and 37/59 = 62.7% response rate in hCAZyme carriers vs non-carriers, respectively; $P = .06$). Finally, in the small group of IBS-D patients (n = 27), *SI*-devoid hCAZyme hypomorphic variants were again significantly associated with increased response to the FODMAP-lowering diet (6/6 = 100% vs 9/21 = 42.9% response

Table 1. hCAZyme Hypomorphics Variants Identified in this Study

Gene	Variant	dbSNP[a]	Ref allele[b]	Alt allele[b]	gnomAD freq[c]	Frequency this study	Carriers diet arm (n = 196)	Carriers OB arm (n = 54)
AMY2B								
	Val190Leu	—	G	T	0.0000009	0.002	—	1
	Val309Ala	rs140209167	T	C	0.0036	0.006	2	1
	Gly319Arg	rs140978983	G	A	0.0315	0.044	16	6
	Ile406Thr	rs151132065	T	C	0.0220	0.018	7	2
LCT								
	Gly928Cys	—	C	A	—	0.002	1	—
	Asn1035Tyr	—	T	A	—	0.004	2	—
MGAM								
	Arg401Cys	rs188481752	C	T	0.0032	0.002	1	—
	Arg637Ser	rs190777514	A	C	0.0019	0.002	1	—
	Leu806Ile	rs956495934	C	A	0.00002	0.002	1	—
	Leu854Phe	rs200141280	C	T	0.0013	0.002	—	1
	Asn858Asp	rs2960746	A	G	0.0001	0.006	3	—
	Cys873Trp	rs768578658	T	G	0.0001	0.002	1	—
	Pro1424Thr	rs185053832	C	A	0.0108	0.010	3	2
	Leu1794Ser	rs201177568	T	C	0.0010	0.002	1	—
MGAM2								
	Ille13Thr	—	T	C	0.000006	0.002	—	1
	Asn606Ser	rs79591013	A	G	0.0122	0.012	4	2
	Cys619Arg	rs774919724	T	C	0.0006	0.002	—	1
	Ala1138Val	—	C	T	—	0.002	1	—
	Leu1174Met	rs73547325	T	A	0.0749	0.052	18	8
	Gln1546Glu	rs7776662	C	G	0.00002	0.002	—	1
	Val2018Ile	—	G	A	—	0.016	8	—
SI								
	Met1Ile	—	C	T	—	0.002	1	—
	Val15Phe	rs9290264	C	A	0.2990	0.340	113	30
	Val371Met	rs138434001	C	T	0.0032	0.004	2	—
	Arg774Gly	rs147207752	T	C	0.0015	0.004	2	—
	Ile799Val	rs150246328	T	C	0.0042	0.004	1	1
	Tyr975His	rs146785675	A	G	0.0055	0.002	1	—
	Ser1490Ile	rs376437234	C	A	0.0001	0.002	—	1
	Leu1520Ter	—	—	A	—	0.008	1	3
TREH								
	Lys140Arg	rs34978247	T	C	0.0069	0.006	3	—
	Met512Thr	rs556006762	A	G	0.0003	0.002	1	—

—, data not available; OB, otilonium bromide.
[a]dbSNP database ID.
[b]Reference and alternative allele.
[c]gnomAD allele frequency in individuals of non-Finnish European ancestry.

rate in hCAZyme carriers vs non-carriers, respectively; $P = .01$). Overall, this indicates hCAZyme findings are not dependent (only) on *SI* genotype.

hCAZyme Genes and Response to Medication

To further explore the relationship between hypomorphic hCAZyme carrier status and response to treatment, we performed additional analyses in the group of patients who were administered OB, that is in a situation where alteration of carbohydrate digestion may not be relevant. Given previous findings in the IBS-D group of patients, where hCAZyme genotype seemed to be most relevant, we selected OB-treated IBS-D patients to run similar analyses. Genomic sequencing identified 15 hypomorphic hCAZyme variants in this group (Table 1). While no significant relationship was observed with the number of affected hCAZyme genes (not shown), OB treatment even proved to be significantly less effective in hypomorphic hCAZyme carriers than non-carriers (Figure 5*B*), thus showing a pattern opposite to the dietary arm of the DOMINO trial.

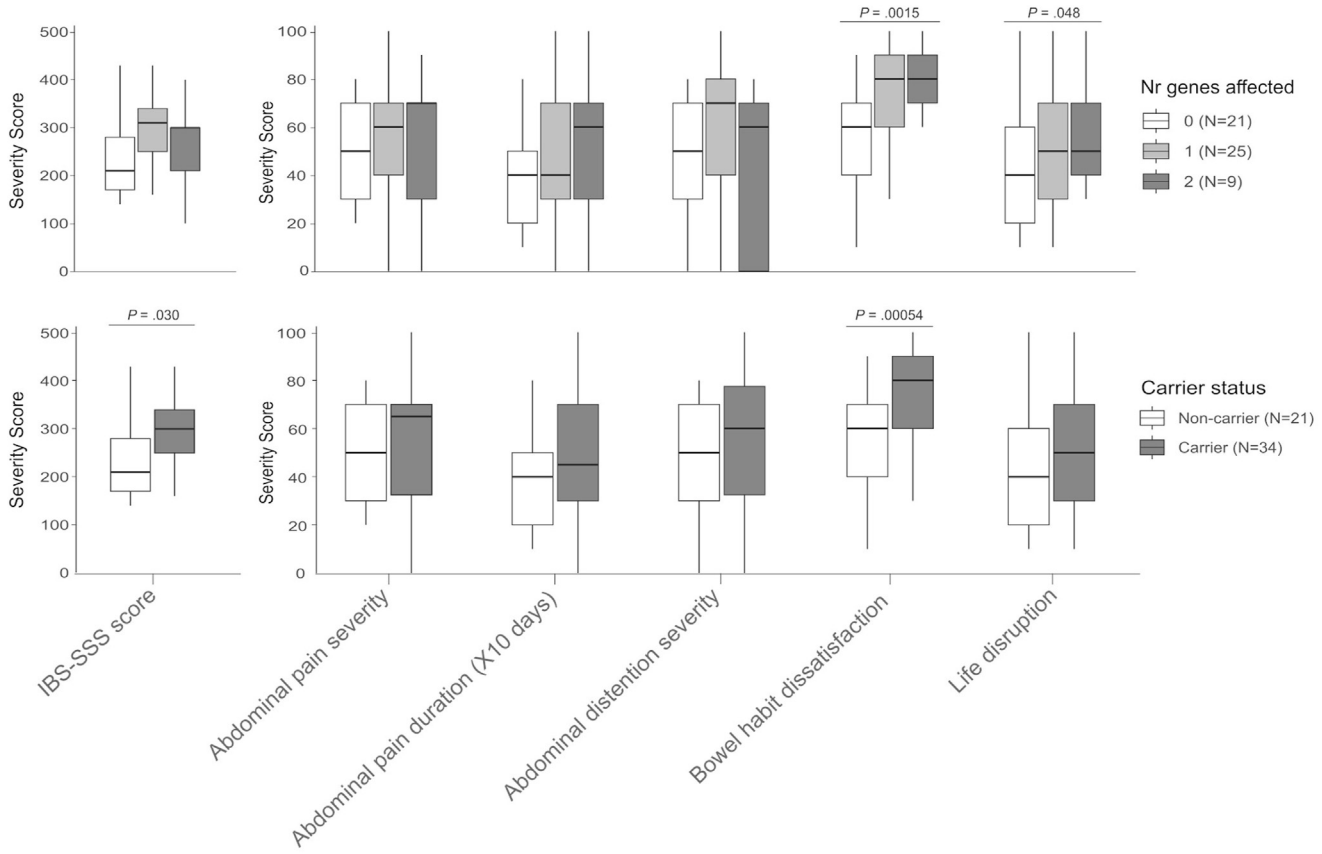

Figure 3. Baseline IBS symptoms scores in 55 IBS-D DOMINO patients from the dietary arm stratified according to genetic variation in the hCAZyme genes. (*Top*) Number of affected hCAZyme genes. (*Bottom*) hCAZyme carrier status. *P* from linear regression adjusted for age + sex (1-tail).

Discussion

We report here a first survey of IBS treatment response in relation to genetic variation in genes involved in the breakdown and digestion of dietary carbohydrates. We show that the hypomorphic hCAZyme genotype is associated with an increased likelihood of responding to a carbohydrate-restricted diet while being irrelevant (if not deleterious) against a treatment based on medication that affects unrelated, different biologic

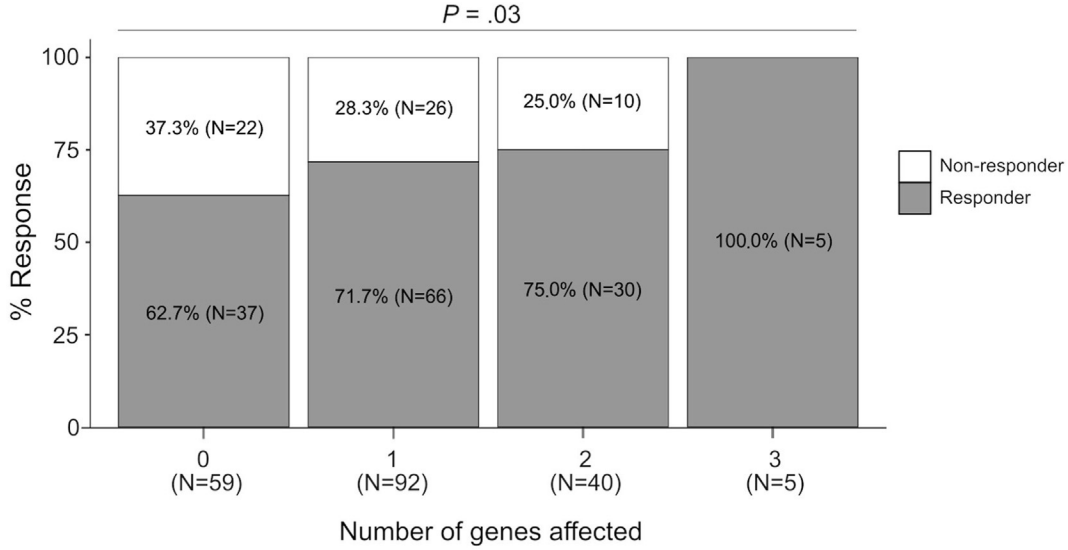

Figure 4. Response to FODMAP-lowering diet in 196 IBS DOMINO patients, stratified according to the number of hCAZyme genes affected. *P* from logistic regression adjusted for age + sex (1-tail).

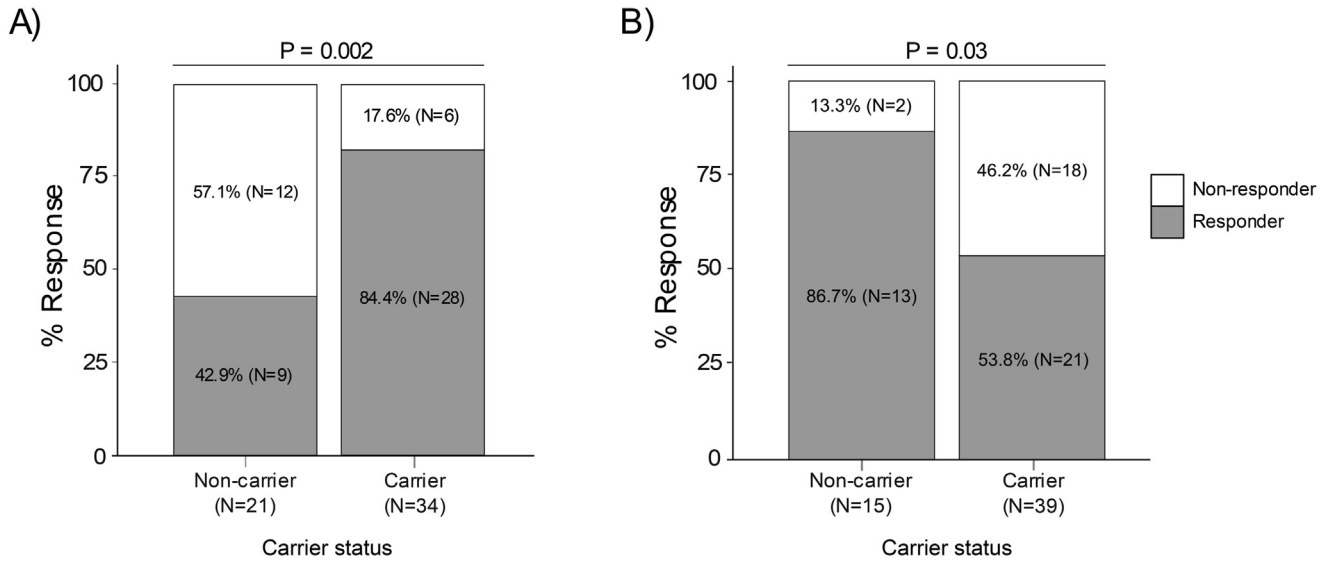

Figure 5. Response to (*A*) FODAMP-lowering diet in 55 IBS-D DOMINO patients and (*B*) OB treatment in 54 IBS-D DOMINO patients, stratified according to hCAZyme carrier status. *P* from logistic regression adjusted for age + sex (1-tail).

mechanisms (OB is an L-type calcium blocker acting as a spasmolytic agent on intestinal smooth muscles).

In a retrospective analysis of the 2-arm (FODMAP and OB) DOMINO interventional trial, we focused our genetic analyses on glycoside hydrolases (EC 3.2.1 enzymes AMY2B, LCT, MGAM, MGAM2, SI, and TREH), the battery of hCAZy enzymes responsible for the digestion of most dietary sugars and starch. Recessive mutations in the corresponding genes are known to cause bowel symptoms in several forms of carbohydrate intolerance (lactose, sucrose, trehalose), due to the migration of undigested saccharides to the lower bowel where they are fermented by resident bacteria inducing gas production, bloating, and diarrhea.[12–14] This represents the foundation of the hypothesis tested in our proof-of-principle study: there may be individuals more sensitive to carbohydrates due to their partially defective digestive enzymes, who benefit more than wild-type carriers from diets restricting carbohydrate intake (like it ensues in the DOMINO FODMAP-reducing diet). While dietary intervention aims to restrict specific carbohydrates by reducing the amount of foods rich in these, there is overlap of hCAZyme substrates across foods, hence a diet focused on a specific carbohydrate or group of carbohydrates will hardly result in their elimination or reduction without also affecting (at least in part) the consumption of others. It is thus conceivable that response to carbohydrate restriction may be more pronounced in individuals who carry hypomorphic variants in multiple hCAZyme genes, and this is indeed the observation made in this study. Of interest, a recent survey showed that a diet based on general carbohydrate restriction is as effective as the low-FODMAP diet in IBS.[23] Our results may be in line with this observation. The FODMAP-lowering diet implemented in the DOMINO trial was essentially used as a prototype for broad (rather than FODMAP-specific) hypothesis testing: it limits the consumption of a series of carbohydrates (FODMAPs and others contained in the restricted foods) that are substrates for hCAZy enzymes predictably defective in hypomorphic variant carriers, and these patients (especially those with multiple hCAZymes affected) seem to benefit more from this dietary restriction than non-carriers. Of note, this evidence was most pronounced in IBS-D, which is a clinical manifestation typical of carbohydrate maldigestion. Moreover, while a large fraction of non-carriers still respond to a carbohydrate-restricted diet, the effect of hCAZyme polymorphism might be underestimated, since our genetic analyses only covered coding variation; it is thus possible that other regulatory variants affect the expression of hCAZy genes, with similar impact on the net capacity to digest carbohydrates (as it happens, for instance, with the persistence genotype and the promoter of the lactase gene).[13] Taken together, these data also suggest that, similar to rare forms of carbohydrate maldigestion, functional changes in CAZyme genes might contribute to generate bowel symptoms via carbohydrate maldigestion in IBS, with initial evidence already recently provided.[24]

If confirmed in follow-up studies, the results reported here may have implications in the management of IBS (especially IBS-D) because of the potential for improving the specificity and efficacy of dietary intervention, providing a rationale for personalized therapeutic (dietary) approaches based on single or multiple hCAZymes gene profiles (and avoidance of specific defective hCAZyme substrates). Indirectly, this may also have positive repercussions on patients' adherence and compliance. As recent surveys show, adherence to a lowFODMAP diet is difficult and requires considerable effort from the patient including several visits to a dietitian, with a significant proportion of IBS patients failing to respond because of non-compliance.[25,26] Prior knowledge of hCAZyme

genotype (and associated likelihood of benefiting from the diet) may thus contribute to decision-making and the ultimate propensity of a patient to agree to adopt this or similar dietary intervention schemes.

The exact mechanisms by which hCAZyme genotype influences the likelihood to respond to a carbohydrate-(FODMAP-) reducing diet was not experimentally verified, because this was beyond the scope of this observational study. However, it is reasonable to assume it is rooted in the accumulation of undigested (poly-, oligo-, and di-) saccharides in the lower bowel of hCAZyme hypomorphic carriers, where they are fermented by the resident bacteria. Indeed, dietary carbohydrates are known to have an important role in shaping the gut microbiota, with bacteria carrying prokaryotic CAZyme genes showing a competitive advantage over others when undigested polysaccharides are abundant in the large bowel.[27] This is relevant to IBS, where the microbiota plays an important role, and where symptoms and their severity have been shown to correlate with bacterial carbohydrate metabolism among other factors.[28–30] Future studies are warranted where the relationship between hCAZyme genotype and gut microbiota composition is investigated.

Additional studies are also needed to elucidate the relationship between individual hCAZyme genes and specific saccharides ultimately impacting the response to dietary treatment, since the relatively small DOMINO sample size and the fact that several individuals were positive at multiple hCAZyme loci did not allow meaningful analyses to be carried out at the single hCAZyme gene level. Data from this study do not replicate the observation of reduced likelihood to respond to a low-FODMAP diet in *SI* hypomorphic carriers,[16] indeed showing evidence in the opposite direction in the DOMINO trial. Among others, factors that may contribute to this difference include: the specific diet adopted (less stringent FODMAP-reducing diet in the DOMINO trial, possibly resulting in the reduction of sucrase-isomaltase substrates too), inclusion criteria for patients enrolment (IBS diagnosed by general practitioners in the DOMINO trial vs Rome III-defined from tertiary centers in the previous randomized controlled trial), the definition of the "response" end point (50-point reduction in IBS-SSS scores in the DOMINO trial compared with "adequate relief" in the previous study), the number of *SI* variants identified and tested (via exome sequencing in the DOMINO trial vs only genotyping in the previous study), the co-occurrence of many *SI* and other hCAZyme hypomorphic variants in DOMINO patients (Supplementary Figure 1 untested in the previous study), and ethnicity (European ancestry vs multiethnic). At the same time, the DOMINO trial did not foresee the use of food diaries allowing to quantify intake of individual carbohydrates. The characterization of specific interactions between hCAZyme genotype and individual carbohydrates in relationship to food restriction is a complex task that spans across a wide spectrum of foods and hCAZyme variant genotypes (including homozygous, heterozygous, compound heterozygous, and polygenic hypomorphic variation, as well as variation in expression levels [not studied here]). This may be addressed in future highly focused studies of hCAZyme gene-carbohydrate interactions, possibly most effectively in re-introduction studies[31–33] in which individual carbohydrates can be tested for their effect on bowel symptoms in hCAZyme genotyped individuals.

An important limitation of this study is the relatively small sample size (especially in the IBS-D group where most significant results were observed), and that findings could not be replicated in independent cohorts. While most CAZyme variants are rare and require suitable sample size to be tested, we are not aware of any other similarly-sized (n = 196 from the dietary arm of the DOMINO study tested here) IBS dietary intervention trial that would allow meaningful validation efforts to be carried out. Future studies can be specifically planned for this purpose. However, it is noteworthy that the analysis of hCAZyme genes in patients with IBS-D from the medication arm did not reveal any association, which serves as an ideal negative control (ie, no association detected when the targeted mechanism is unrelated to CAZYme function).

Finally, the establishment of scalable, sensitive, functional assays for hCAZyme relative gene variant activity quantification is needed, because past and current genetic research is mostly based on computational predictions that require validation in reproducible high-throughput *in vitro* systems.

In conclusion, we provide initial evidence that functional (hypomorphic) hCAZymes variations collectively influence the likelihood of responding to dietary treatment based on restricting the intake of certain carbohydrates. This information may be relevant to increase therapeutic precision and efficacy via genotype-driven dietary intervention in IBS. Future studies can be designed to consolidate this evidence with replication in independent cohorts and to obtain biologic insight at the mechanistic level.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2024.09.004.

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Conflicts of interest

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Supplementary Methods

Selection of hCAZyme Genes and Genomic Sequencing

The annotation of the molecular pathways *Carbohydrate digestion and absorption* (https://www.kegg.jp/entry/map04973) and *Starch and sucrose metabolism* (https://www.kegg.jp/entry/map00500) from the Kyoto Encyclopedia of Genes and Genomes was used to select hCAZyme genes to be included in an IBS-focused targeted next-generation sequencing panel (see Methods). Upon implementation of this assay to sequence DOMINO participants' DNA (see later), high-coverage/high-quality next-generation sequencing data could be obtained for the hCAZyme genes *AMY2B*, *LCT*, *MGAM*, *MGAM2*, *SI*, and *TREH*, which were included in downstream analyses.

Targeted Sequencing of hCAZyme Genes

An Illumina AmpliSeq DNA targeted next-generation sequencing assay focused on IBS-candidate genes was designed using Illumina Design Studio, which included 10 hCAZyme genes (*AMY1A*, *AMY1B*, *AMY1C*, *AMY2A*, *AMY2B*, *LCT*, *MGAM*, *MGAM2*, *SI*, *TREH*). Panel sequencing was carried out on DNA (blood samples) from selected DOMINO participants and, for the purpose of this study, high-quality sequencing data were further analyzed for 6 genes (*AMY2B*, *LCT*, *MGAM*, *MGAM2*, *SI*, and *TREH)* whose entire coding sequence (all exons and exon boundaries) was successfully covered (>90% of the target sequence with 30x coverage). Sequencing of 2 x 150 bp paired-end reads was performed on an Illumina NextSeq, and the reads mapped to the reference human genome build GRCh38 using the IKMB exome pipeline (https://github.com/ikmb/exome-seq). Variant calling was performed using DeepVariant (v.1.0.)[1] and Genome Analysis Toolkit,[2] in parallel and only variants called by both tools were included in the analyses.[3] Targeted sequencing data were further validated by whole exome sequencing on 5 individuals randomly selected among DOMINO participants, obtaining identical results. ANNOVAR[4] was used for functional annotation of hCAZyme variants, to filter for coding missense (non-synonymous and/or stop-gain/loss) changes, before assigning pathogenicity scores (see later). Global screening array genotyping data available for DOMINO participants[5] was used for cohort-level quality controls, filtering our individuals of non-European ancestry, with missing data, heterozygosity rate >6SD, and/or calling rate <0.85.

Identification of hCAZyme Hypomorphic Variants

To identify hCAZyme hypomorphic variants (defective variants with absent/reduced enzymatic activity), the functional relevance of non-synonymous changes was predicted in silico using a combination of mendelian-clinically applicable pathogenicity (http://bejerano.stanford.edu/MCAP) and combined annotation-dependent depletion (http://cadd.gs.washington.edu/) computational tools, as previously described.[6-8] These bioinformatic approaches were used because of their documented power to predict deleteriousness (pathogenicity) of DNA substitutions for clinical utility.[9,10] Because of demonstrated superior performance,[9] mendelian-clinically applicable pathogenicity M-CAP scores were prioritized when available (for rare coding variants with an allele frequency <1%), while combined annotation-dependent depletion scores were adopted for all remaining variants.

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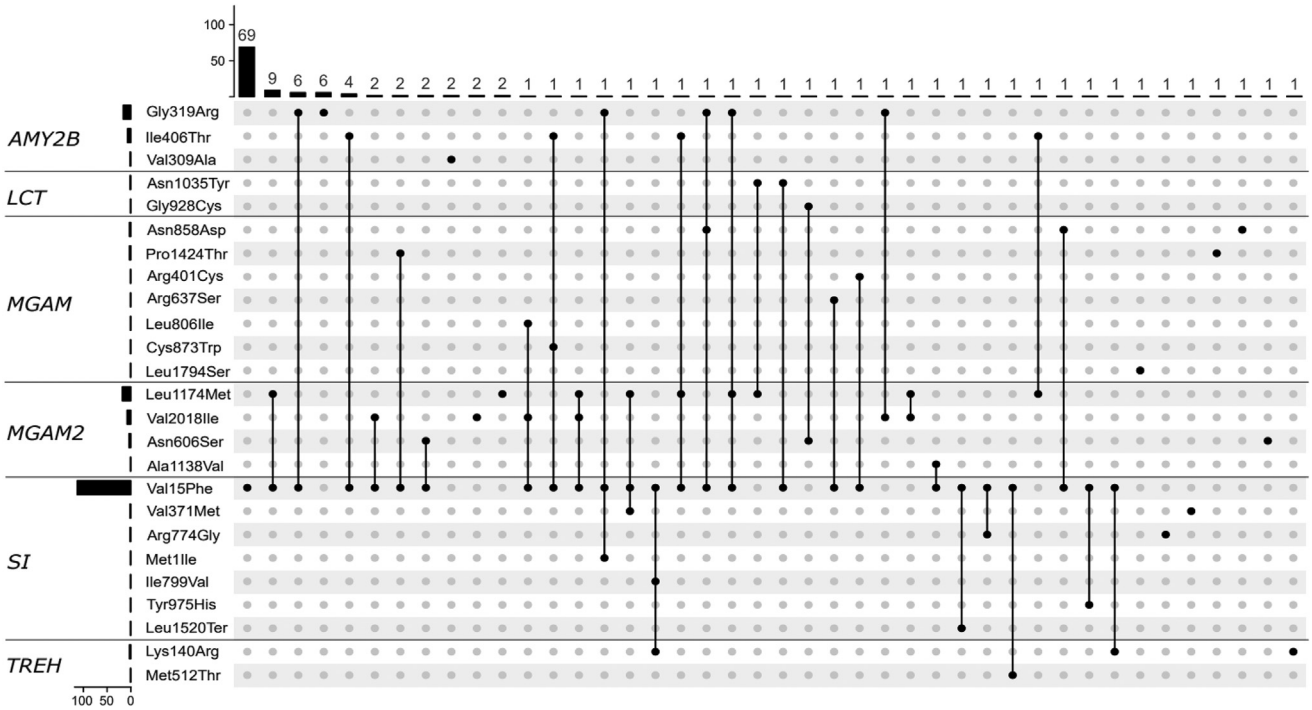
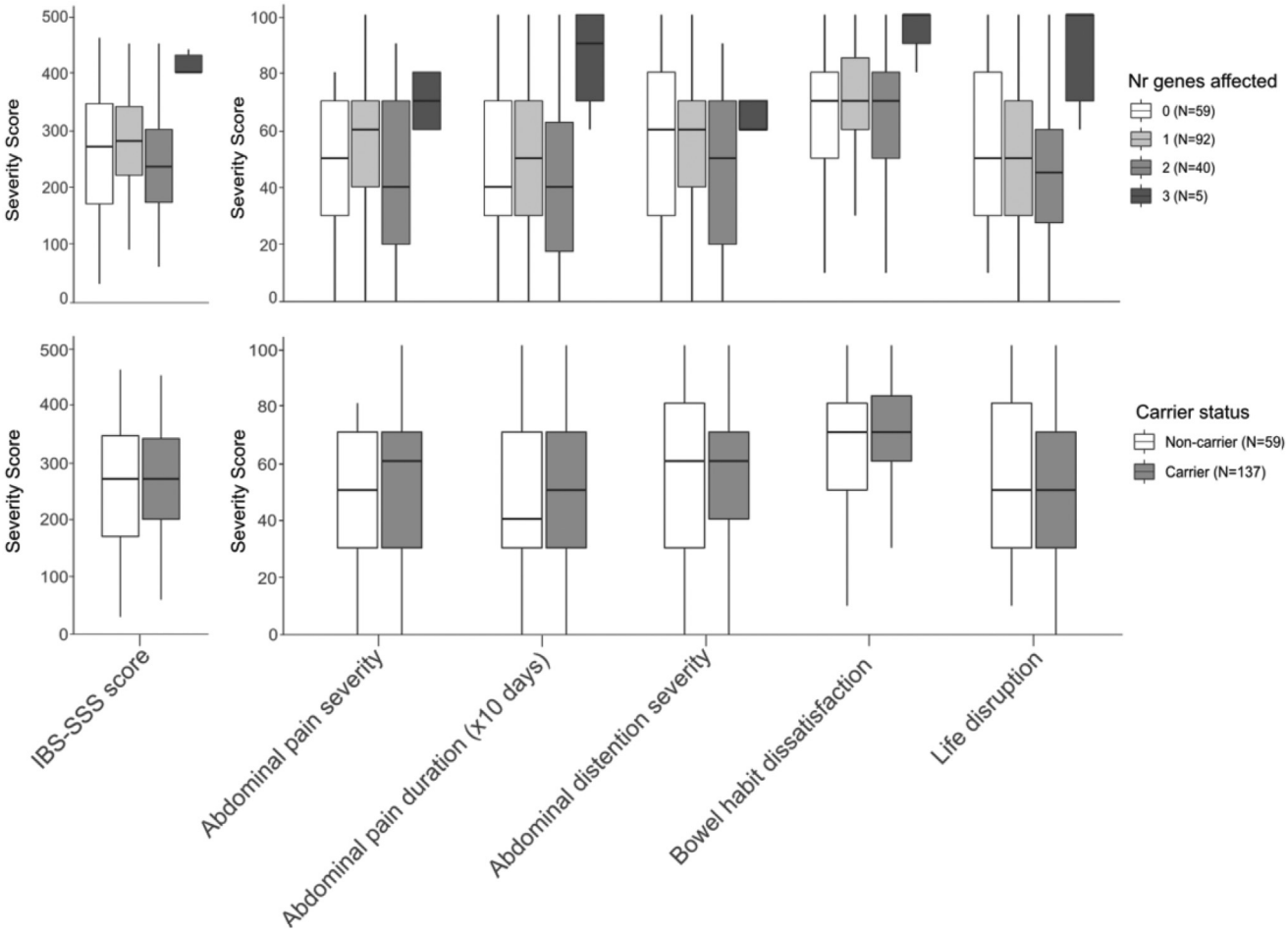
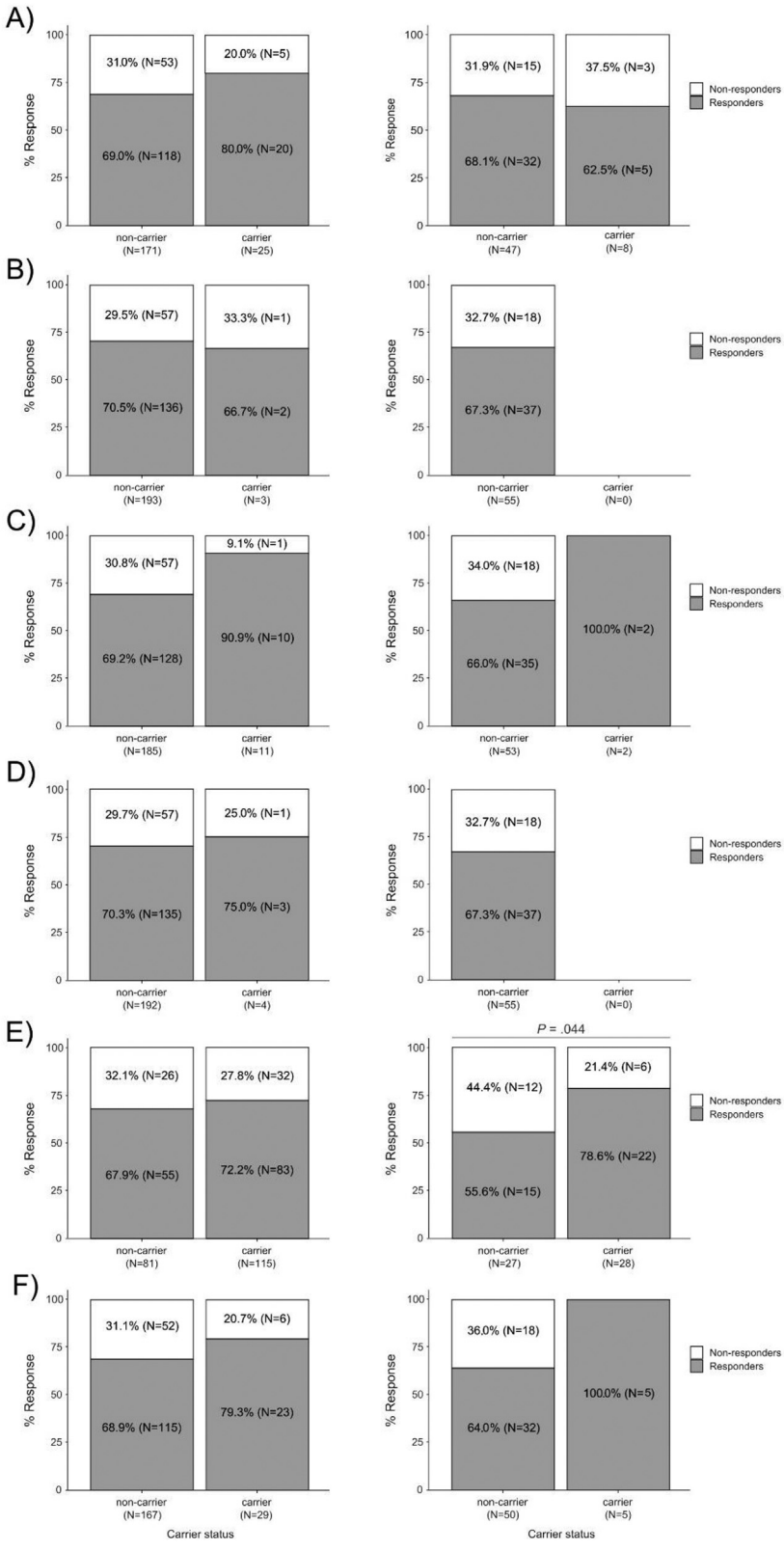


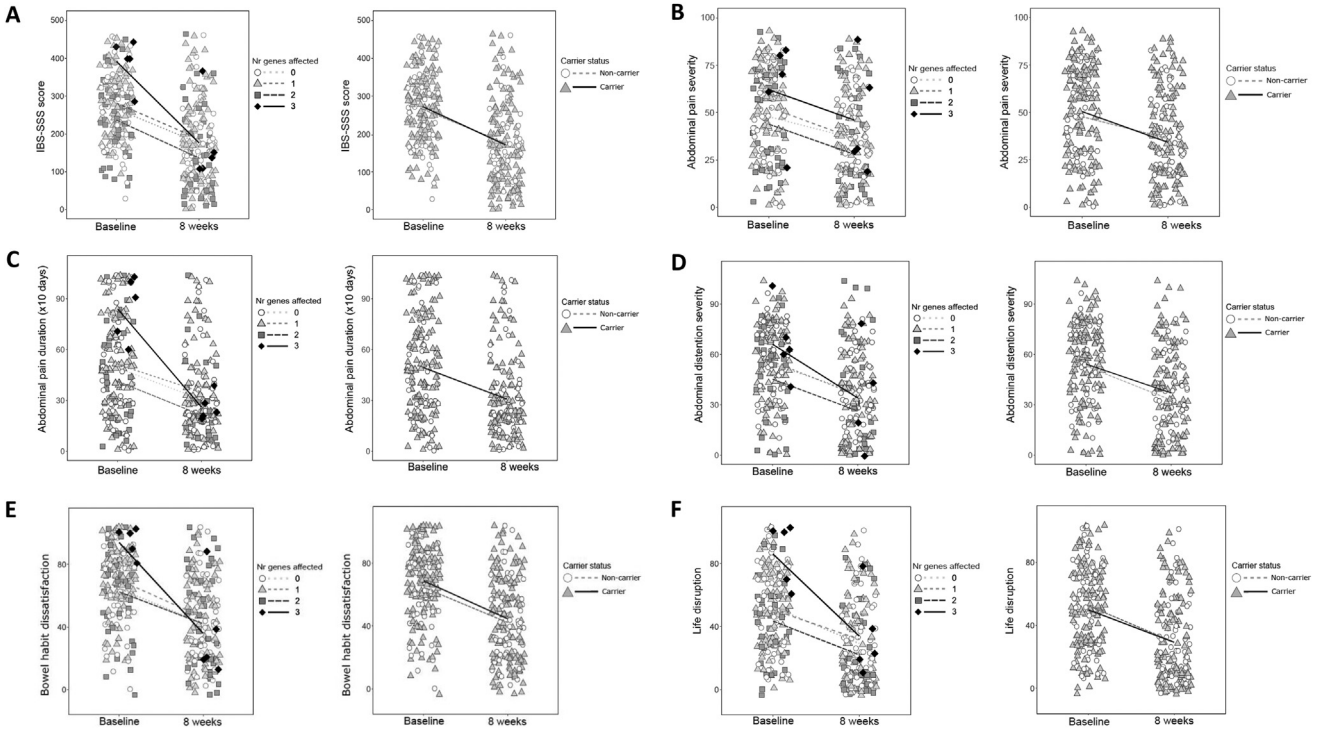
Supplementary Figure 1. UpSet plot showing all genotypic combinations of hypomorphic variants present in hCAZyme genes from this study.



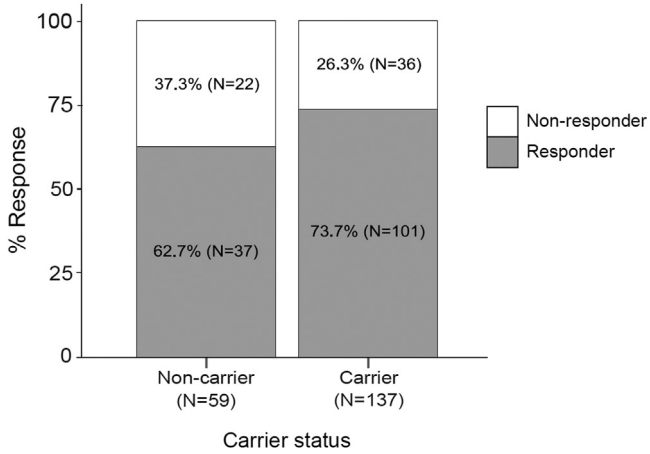
Supplementary Figure 2. Baseline IBS symptoms in 196 IBS DOMINO patients from the dietary arm stratified according to genetic variation in the hCAZyme genes. (*Top*) number of affected hCAZyme genes. (*Bottom*) hCAZyme carrier status.



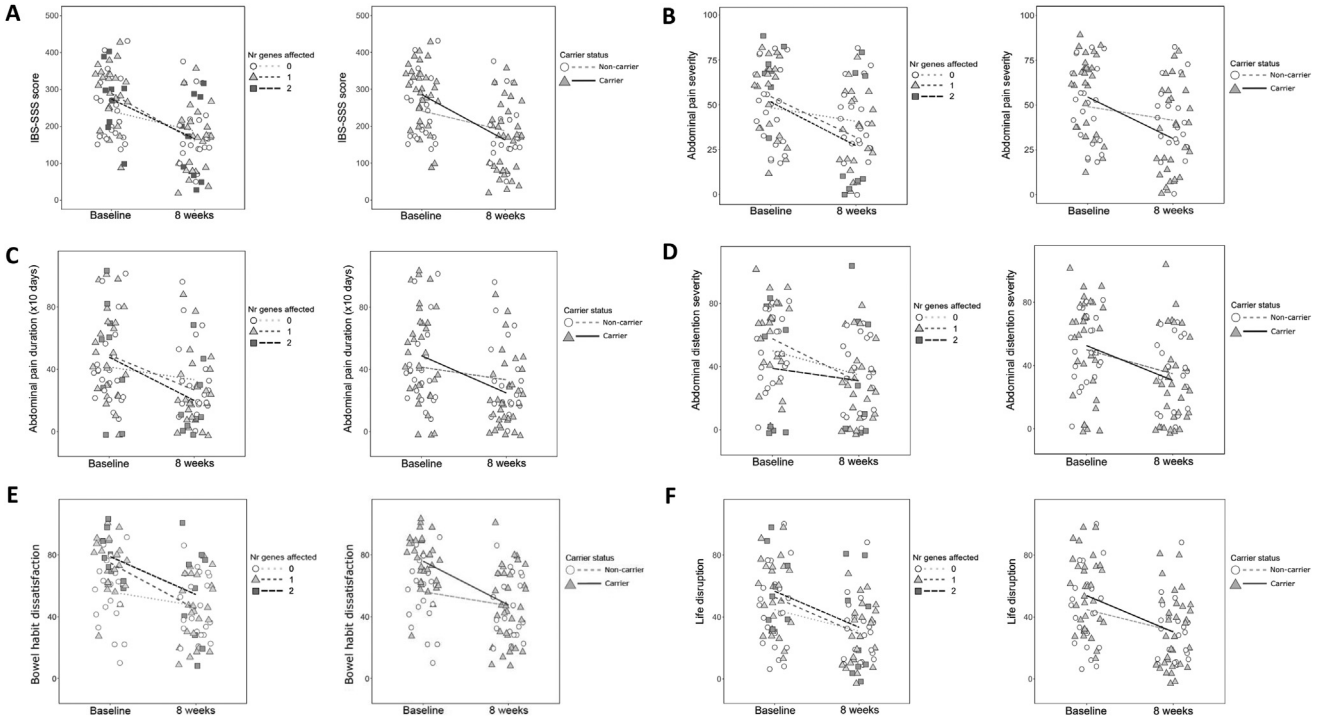
Supplementary Figure 3. Response to a FODMAP-lowering diet in 196 IBS DOMINO patients (*left*) and 55 IBS-D DOMINO patients (*right*) stratified according to carrier status (non-carrier or carrier) for each hCAZyme gene. (*A*) *AMY2B*, (*B*) *LCT*, (*C*) *MGAM*, (*D*) *TREH*, (*E*) *SI*, and (*F*) MGAM2. *P* from Fisher exact test (1-tail).



Supplementary Figure 4. Variation of IBS symptoms after 8 weeks of treatment in 196 IBS patients from the dietary arm. Total (*A*) and individual (*B-F*) symptom scores are stratified according to genetic variation in the hCAZyme genes: (*left*) number of affected hCAZyme genes; (*right*) hCAZyme carrier status.



Supplementary Figure 5. Response to a FODMAP-lowering diet in 196 IBS DOMINO patients stratified according to hCAZyme carrier status.



Supplementary Figure 6. Variation of IBS symptoms after 8 weeks of treatment in 55 IBS-D patients from the dietary arm. Total (*A*) and individual (*B-F*) symptom scores are stratified according to genetic variation in the hCAZyme genes: (*left*) number of affected hCAZyme genes; (*right*) hCAZyme carrier status.

Supplementary Table 1. Demographic and Clinical Characteristics of Patients Included in the Study

	Diet[a]	Medication[a]
Patients, n	196	54
Females, n (%)	147 (75.0)	38 (70.4)
Age, mean ± SD	41.0 ± 14.7	37.5 ± 14.3
Response, n (%)	138 (70.4)	34 (63.0)
Stool type, n (%)		
IBS-D	55 (28.1)	54 (35.3)
IBS-M	73 (37.2)	—
IBS-C	39 (19.9)	—
IBS-U	29 (14.8)	—
Symptoms at baseline, mean ± SD		
IBS-SSS score	270.4 ± 94.1	262.0 ± 97.0
Abdominal distention severity	53.4 ± 25.9	46.7 ± 28.1
Life disruption	50.6 ± 26.1	52.8 ± 25.2
Abdominal pain severity	49.6 ± 24.7	51.5 ± 24.4
Bowel habit dissatisfaction	67.5 ± 23.4	66.5 ± 26.0
Abdominal pain duration (x10 d)	4.9 ± 3.0	4.5 ± 2.9
Symptoms after 8 wk treatment, mean ± SD		
IBS-SSS score	172.5 ± 111.2	173.1 ± 99.7
Abdominal distention severity	34.1 ± 27.3	33.9 ± 27.0
Life disruption	29.3 ± 26.9	31.0 ± 23.4
Abdominal pain severity	34.8 ± 24.7	37.2 ± 23.2
Bowel habit dissatisfaction	43.9 ± 27.0	43.7 ± 23.8
Abdominal pain duration (x10 d)	3.1 ± 2.6	3.3 ± 2.3

IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with mixed type; IBS-SSS, IBS-Symptom Severity Score; IBS-U, IBS unclassified; SD, standard deviation.

[a]No significant difference detected for any of the variables reported.

Supplementary Table 2. hCAZyme Variants Identified in this Study

Gene	Variant	BP (GRCh 38)	dbSNP	Ref allele	Alt allele	gnomAD freq	Freq this study	M-CAP	M-CAP pathogenicity	CADD	CADD pathogenicity	Hypomorphic	Carriers diet arm (n = 196)	Carriers OB arm (n = 54)
AMY														
	Val190Leu	chr1:103573762	—	G	T	0.0000009	0,002	0.139	1	—	—	1	—	1
	Val309Ala	chr1:103575270	rs140209167	T	C	0.0025	0.006	0.170	1	—	—	1	2	1
	Gly319Arg	chr1:103575299	rs140978983	G	A	0.0315	0.044	—	—	27.3	1	1	16	6
	Arg358His	chr1:103575512	rs150608402	G	A	0.0002	0.004	0.003	0	—	—	0	2	—
	Ile406Thr	chr1:103577605	rs151132065	T	C	0.0220	0.018	—	—	26.0	1	1	7	2
LCT														
	Ala152T	chr2:135836716	rs114525655	C	T	0.0001	0.002	—	—	16.7	0	0	—	1
	Val219Ile	chr2:135833176	rs3754689	C	T	0.1350	0.176	—	—	10.1	0	0	66	14
	Ile362Val	chr2:135817964	rs4954449	T	C	0.9999	0.998	—	—	13.8	1	0	196	54
	Phe530Leu	chr2:135817460	—	A	G	0.000001	0.002	0.014	0	—	—	0	1	—
	Gly928Cys	chr2:135809565	—	C	A	—	0.002	0.127	1	—	—	1	1	—
	Asn1035Tyr	chr2:135809244	—	T	A	—	0.004	0.135	1	—	—	1	2	—
	Ala1096T	chr2:135809061	rs146467199	C	T	0.0009	0.002	0.006	0	—	—	0	—	1
	Thr1329Met	chr2:135807315	rs555708380	G	A	0.0000	0.002	0.010	0	—	—	0	1	—
	Ala1483Ser	chr2:135804784	rs139591272	C	A	0.0009	0.002	0.015	0	—	—	0	1	—
	Tyr1549Cys	chr2:135803947	rs147495948	T	C	0.0005	0.004	0.007	0	—	—	0	2	—
	Asn1639Ser	chr2:135798089	rs2322659	T	C	0.7803	0.736	—	—	2.6	0	0	179	50
MGAM														
	Ile25Val	chr7:142005603	rs61733478	A	G	0.00004	0.002	—	—	12.9	0	0	1	—
	Ile28Thr	chr7:142005613	rs201144916	T	C	0.0035	0.004	—	—	13.5	0	0	2	—
	Asp391Asn	chr7:142027685	rs184092742	G	A	0.0061	0.008	—	—	13.9	0	0	4	—
	Arg401Cys	chr7:142027715	rs188481752	C	T	0.0032	0.002	0.066	1	—	—	1	1	—
	Gln404His	chr7:142027726	rs2272330	G	T	0.0091	0.004	—	—	11.0	0	0	1	1
	Arg637Ser	chr7:142034793	rs190777514	A	C	0.0019	0.002	0.133	1	—	—	1	1	—
	His755Tyr	chr7:142038562	rs113689539	C	T	0.0001	0.004	—	—	14.5	0	0	1	1
	Leu806Ile	chr7:142040764	rs956495934	C	A	0.00002	0.002	0.037	1	—	—	1	1	—
	Leu854Phe	chr7:142047846	rs200141280	C	T	0.0013	0.002	0.029	1	—	—	1	—	1
	Asn858Asp	chr7:142047858	rs2960746	A	G	0.0001	0.006	—	—	21.2	1	1	3	—
	Cys873Trp	chr7:142050266	rs768578658	T	G	0.0001	0.002	0.059	1	—	—	1	1	—
	Thr907Met	chr7:142050779	rs187898444	C	T	0.0117	0.010	—	—	0.2	0	0	2	3
	Glu976Ala	chr7:142052415	rs116536012	A	C	0.0258	0.030	—	—	10.6	0	0	10	5
	Arg1039His	chr7:142052941	rs139662456	G	A	0.0001	0.002	—	—	7.5	0	0	—	1
	Pro1057Ser	chr7:142054763	rs780243925	C	T	0.00001	0.002	0.007	0	—	—	0	1	—
	Pro1424Thr	chr7:142063511	rs185053832	C	A	0.0108	0.010	—	—	25.0	1	1	3	2
	Ile1783Val	chr7:142103290	rs140217455	A	G	0.0031	0.006	0.011	0	—	—	0	1	2
	Leu1794Ser	chr7:142103324	rs201177568	T	C	0.0010	0.002	0.049	1	—	—	1	1	—

Supplementary Table 2. Continued

Gene	Variant	BP (GRCh 38)	dbSNP	Ref allele	Alt allele	gnomAD freq	Freq this study	M-CAP	M-CAP pathogenicity	CADD	CADD pathogenicity	Hypomorphic	Carriers diet arm (n = 196)	Carriers OB arm (n = 54)
MGAM2														
	Ille13Thr	chr7:142116911	—	T	C	0.000006	0.002	0.170	1	—	—	1	—	1
	Phe327Leu	chr7:142138562	rs6464465	C	G	0.3588	0.380	—	—	17.8	0	0	126	30
	Glu471Lys	chr7:142143862	rs76786761	G	A	0.0598	0.074	—	—	11.9	0	0	27	8
	Pro590Ser	chr7:142154151	rs73158444	C	T	0.0891	0.082	—	—	18.3	0	0	30	8
	Asn606Ser	chr7:142154739	rs79591013	A	G	0.0122	0.012	—	—	25.9	1	1	4	2
	Cys619Arg	chr7:142154777	rs774919724	T	C	0.0006	0.002	0.050	1	—	—	1	—	1
	Arg682Trp	chr7:142158057	rs567505779	C	T	0.0028	0.002	0.010	0	—	—	0	1	—
	Ser745Leu	chr7:142160147	rs114108719	C	T	0.0001	0.002	—	—	11.4	0	0	—	1
	Thr1096Met	chr7:142171376	rs111852582	C	T	0.00001	0.002	—	—	18.2	0	0	1	
	Ala1138Val	chr7:142172159	—	C	T	—	0.002	0.063	1	—	—	1	1	—
	Leu1174Met	chr7:142172723	rs73547325	T	A	0.0749	0.052	—	—	25.0	1	1	18	8
	Thr1326Ala	chr7:142185128	rs201188036	A	G	0.0028	0.006	0.002	0	—	—	0	1	2
	Pro1425Leu	chr7:142189433	rs563508674	C	T	0.00005	0.002	0.003	0	—	—	0	1	—
	Val1430Met	chr7:142189447	rs4726494	G	A	0.0087	0.014	0.013	0	—	—	0	7	—
	Gln1546Glu	chr7:142197403	rs7776662	C	G	0.00002	0.002	—	—	27.0	1	1	—	1
	Arg1589Gln	chr7:142197533	—	G	A	0.000003	0.002	0.010	0	—	—	0	—	1
	Val2018Ile	chr7:142220563	—	G	A	—	0.016	0.090	1	—	—	1	8	—
	Ser2108Asn	chr7:142220834	rs868461091	G	A	0.00005	0.002	0.019	0	—	—	0	1	—
	Ile2384Phe	chr7:142221661	rs114133571	A	T	0.0002	0.002	—	—	11.2	0	0	1	—
	Pro2390Leu	chr7:142221680	rs60502652	C	T	0.0861	0.080	—	—	11.9	0	0	32	8
SI														
	Met1Ile	chr3:165076010	—	C	T	—	0.002	0.841	1	—	—	1	1	—
	Val15Phe	chr3:165075970	rs9290264	C	A	0.2990	0.340	—	—	24.5	1	1	113	30
	Glu40Gly	chr3:165074667	rs747623135	T	C	0.00002	0.002	0.012	0	—	—	0	1	—
	Ser186Pro	chr3:165067419	rs142447888	A	G	0.0003	0.002	0.010	0	—	—	0	1	—
	Thr231Ala[a]	chr3:165065377	rs9283633	T	C	0.5889	0.080	—	—	12.8	0	0	—	—
	Val371Met	chr3:165059937	rs138434001	C	T	0.0032	0.004	0.412	1	—	—	1	2	—
	Arg774Gly	chr3:165038006	rs147207752	T	C	0.0015	0.004	0.113	1	—	—	1	2	—
	Ile799Val	chr3:165037931	rs150246328	T	C	0.0042	0.004	0.058	1	—	—	1	1	1
	Tyr975His	chr3:165023746	rs146785675	A	G	0.0055	0.002	—	—	25.1	1	1	1	—
	Ser1490Ile	chr3:164998611	rs376437234	C	A	0.0001	0.002	0.053	1	—	—	1	—	1
	Met1523Ile	chr3:164996744	rs4855271	C	T	0.9072	0.008	—	—	9.6	0	0	196	53
	Leu1520Ter	chr3:164996758	—	—	A	—	0.008	—	—	—	—	1	1	3

Supplementary Table 2. Continued

Gene	Variant	BP (GRCh 38)	dbSNP	Ref allele	Alt allele	gnomAD freq	Freq this study	M-CAP	M-CAP pathogenicity	CADD	CADD pathogenicity	Hypomorphic	Carriers diet arm (n = 196)	Carriers OB arm (n = 54)
TREH														
	Lys140Arg	chr11:11866288	rs34978247	T	C	0.0069	0.006	—	—	20.6	1	1	3	—
	Ile328Thr	chr11:11866065	rs200440695	A	G	0.0052	0.004	0.004	0	—	—	0	2	—
	Thr389Ala	chr11:11865990	rs2276065	T	C	0.2298	0.210	—	—	1.1	0	0	64	24
	Tyr449His	chr11:118659457	rs11827611	A	G	0.00005	0.004	—	—	4.8	0	0	—	2
	Arg486Trp	chr11:11865899	rs2276064	G	A	0.0129	0.008	—	—	16.7	0	0	4	—
	Phe492Leu	chr11:118658976	rs374204865	A	G	0.0001	0.002	0.025	0	—	—	0	—	1
	Met512Thr	chr11:11865891	rs556006762	A	G	0.0003	0.002	0.117	1	—	—	1	1	—
	His566Tyr	chr11:11865834	rs200772007	G	A	0.0005	0.004	0.004	0	—	—	0	1	1

—, data not available; Variant, corresponding amino acid change; Alt Allele, alternative allele according to gnomAD; BP (GRCh38), genomic position, based on human genome reference GRCh38; bSNP, dbSNP database ID; CADD, Combined Annotation Dependent Depletion phred-like scores (https://cadd.gs.washington.edu/); Freq this study, allele frequency in 250 IBS DOMINO samples from dietary and medication arm; gnomAD freq, allele frequency in individuals of non-Finnish European ancestry as from gnomAD; M-CAP, Mendelian Clinically Applicable Pathogenicity (M-CAP) pathogenicity likelihood scores (http://bejerano.stanford.edu/mcap/); M-CAP pathogenicity, whether the variant is predicted to be pathogenic (1) or benign (0) based on M-CAP pathogenicity score >0.025; Ref allele, reference allele according to gnomAD v3.1.1 data (https://gnomad.broadinstitute.org/); CADD pathogenicity, whether the variant is predicted to be pathogenic (1) or benign (0) based on CADD pathogenicity score >20; Hypomorphic, whether the variant is predicted to be pathogenic (1) or benign (0) based on MCAP and CADD; Carriers diet arm (n = 196), number of carriers among 196 IBS patients from the dietary arm; Carriers OB arm (n = 54), number of carriers among 54 IBS-D patients from the otilonium bromide arm.

[a]Thr231Ala was detected but failed to generate reliable results (not adequately covered), although this is irrelevant to the aim of this study (this is not a hypomorphic variant).

Supplementary Table 3. Demographics and Symptom Scores in hCAZyme Carrier Groups from the Dietary Arm

	Carriers	Non-carriers	*P* value
Patients, n	137	59	
Females, n (%)	102 (74.5)	45 (76.3)	ns
Age, mean ± SD	40.8 ± 14.5	41.5 ± 15.2	ns
Stool type, n (%)			
IBS-D	34 (24.8)	21 (35.6)	ns
IBS-M	50 (36.5)	23 (39.0)	ns
IBS-C	31 (22.6)	8 (13.6)	ns
IBS-U	22 (16.1)	7 (11.9)	ns
Symptoms at baseline, mean ± SD			
IBS-SSS score	272.3 ± 90.7	265.9 ± 102.1	ns
Abdominal distention severity	53.0 ± 26.3	54.2 ± 25.3	ns
Life disruption	49.8 ± 25.6	52.5 ± 27.4	ns
Abdominal pain severity	50.4 ± 25.2	47.7 ± 23.6	ns
Bowel habit dissatisfaction	68.7 ± 23.0	64.6 ± 24.3	ns
Abdominal pain duration (x10 d)	4.9 ± 2.9	4.9 ± 3.1	ns

IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with mixed type; IBS-SSS, IBS-Symptom Severity Score; IBS-U, IBS unclassified; ns, not significant; SD, standard deviation.