Increased Prevalence of Rare Sucrase-isomaltase Pathogenic Variants in Irritable Bowel Syndrome Patients

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Patients with irritable bowel syndrome (IBS) often associate their symptoms to certain foods. In congenital sucrase-isomaltase deficiency (CSID), recessive mutations in the *SI* gene (coding for the disaccharidase digesting sucrose and 60% of dietary starch)[1] cause clinical features of IBS through colonic accumulation of undigested carbohydrates, triggering bowel symptoms.[2] Hence, in a previous study,[3] we hypothesized that CSID variants reducing SI enzymatic activity may contribute to development of IBS symptoms. We detected association with increased risk of IBS for 4 rare loss-of-function variants typically found in (homozygous) CSID patients, because carriers (heterozygous) of these rare variants were more common in patients than in controls.[1,4] Through a 2-step computational and experimental strategy, the present study aimed to determine whether other (dys-)functional *SI* variants are associated with risk of IBS in addition to known CSID mutations. We first aimed to identify all *SI* rare pathogenic variants (SI-RPVs) on the basis of integrated Mendelian Clinically Applicable Pathogenicity (M-CAP) and Combined Annotation Dependent Depletion

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Abbreviations used in this paper: CADD, Combined Annotation Dependent Depletion; CSID, congenital sucrase-isomaltase deficiency; ExAC, Exome Aggregation Consortium; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; M-CAP, Mendelian Clinically Applicable Pathogenicity; SI-RPV, *SI* rare pathogenic variants.

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Table 1. Prevalence of SI-RPVs in IBS Patients and ExAC Reference Individuals

SNP	Reference allele	RPV	Amino acid change	M-CAP score	CADD score	SI-RPV carriers					
						IBS (N = 2207)	IBS-C (N = 598)	IBS-D (N = 952)	IBS-M (N = 503)	IBS-U (N = 154)	ExAC (N = 33,370)
						N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
rs200745562	G	A	p.Arg250Cys	0.152		3 (0.14)	1 (0.17)	2 (0.21)	—	—	15 (0.04)
rs77546399	G	A	p.Pro348Leu	0.417		12 (0.54)	4 (0.67)	6 (0.63)	1 (0.20)	1 (0.65)	149 (0.45)
rs138434001	C	T	p.Val371Met	0.412		8 (0.36)	2 (0.33)	1 (0.11)	4 (0.80)	1 (0.65)	153 (0.46)
rs142789249	T	C	p.Glu640Gly	0.039		2 (0.09)	1 (0.17)	1 (0.11)	—	—	18 (0.05)
rs188320908	A	T	p.Val717Asp	0.308		1 (0.05)	1 (0.17)	—	—	—	7 (0.02)
rs147207752	T	C	p.Arg774Gly	0.113		10 (0.45)	5 (0.84)	4 (0.42)	1 (0.20)	—	79 (0.24)
rs140230726	A	G	p.Tyr867His	0.142		1 (0.05)	—	—	1 (0.20)	—	14 (0.04)
rs146785675	A	G	p.Tyr975His		26.6	36 (1.63)	13 (2.17)	17 (1.79)	4 (0.80)	2 (1.30)	382 (1.14)
rs200451408	G	A	p.Arg1124Stop		37	1 (0.05)	—	1 (0.11)	—	—	8 (0.02)
rs78013297	G	A	p.Pro1200Ser	0.389		1 (0.05)	—	1 (0.11)	—	—	1 (0.003)
rs143388292	T	C	p.Arg1367Gly	0.17		2 (0.09)	—	2 (0.21)	—	—	28 (0.08)
rs145734588	C	T	p.Glu1414Lys	0.075		3 (0.14)	—	1 (0.11)	2 (0.40)	—	8 (0.02)
rs142090504	A	C	p.Tyr1417Stop		36	1 (0.05)	—	—	—	1 (0.65)	6 (0.02)
rs145246112	C	T	p.Arg1484His	0.293		1 (0.05)	—	—	—	1 (0.65)	19 (0.06)
rs149414344	A	C	p.Phe1625Val	0.057		1 (0.05)	—	1 (0.11)	—	—	1 (0.003)
rs142018224	C	G	p.Val1667Leu	0.032		2 (0.09)	—	1 (0.11)	1 (0.20)	—	20 (0.06)
rs145556619	C	A	p.Gly1760Val	0.204		3 (0.14)	—	2 (0.21)	1 (0.20)	—	20 (0.06)
Total						88 (3.99)	27 (4.51)	40 (4.20)	15 (2.98)	6 (3.90)	928 (2.78)
P value						***.00049***	***.0055***	***.0045***	.39	.21	
Odds ratio (95% confidence interval)						***1.45 (1.16–1.81)***	***1.65 (1.12–2.44)***	***1.53 (1.11–2.12)***	1.07 (0.64–1.80)	0.71 (0.31–1.60)	

CADD, Combined Annotation Dependent Depletion; ExAC, Exome Aggregation Consortium; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with alternating constipation and diarrhea; IBS-U, unsubtyped IBS; M-CAP, Mendelian Clinically Applicable Pathogenicity; SI-RPV, sucrase-isomaltase rare pathogenic variants; SNP, single nucleotide polymorphism.

(CADD) predictive (clinically relevant) scores; next, we inspected genotype data currently available for 2207 IBS patients from a large ongoing project to compare SI-RPV case frequencies with ethnically matched population frequencies from the Exome Aggregation Consortium (ExAC).

Methods

Study Subjects

A total of 2207 IBS patients (598 IBS with constipation [IBS-C], 952 IBS with diarrhea [IBS-D], 503 IBS with alternating constipation and diarrhea, and 154 unsubtyped IBS according to Rome Criteria) of European ancestry were included on the basis of available genotype data from the bellygenes initiative study (www.bellygenes.org). On approval from local ethical committees, IBS patients were recruited at tertiary centers in Sweden, The Netherlands, Belgium, Italy, and United States as described in detail in previous publications, including former genetic studies of IBS.[5–8] Ethnically matched (non-Finnish, European ancestry; N = 33,370) reference population frequency of relevant SI-RVPs were extracted from ExAC (http://exac.broadinstitute.org).

Selection of Sucrase-isomaltase Rare Pathogenic Variants

An inventory of all *SI* rare variants (minor allele frequency <1%) was created by extracting single nucleotide polymorphism data from dbSNP (http://www.ncbi.nlm.nih.gov/snp/). Sequential data processing with M-CAP (http://bejerano.stanford.edu/MCAP) and CADD (http://cadd.gs.washington.edu/) was then performed to identify and select SI-RPVs. These computational resources were used because of their documented power to predict deleteriousness (pathogenicity) of DNA substitutions for clinical utility, assigning priority to M-CAP scores (pathogenicity cutoff >0.025, 5% misclassification rate) over CADD scores (pathogenicity cutoff >0.20, 26% misclassification rate).

Genotype Quality Control and Statistical Analysis

Before extraction of SI-RPV data, stringent quality control filters were applied to available IBS patients' Illumina HumanCoreExome genotype data, including per-sample and per-marker success rate, relatedness, and removal of population outliers based on principal component analysis. To avoid uncertainty, only observed (not imputed) SI-RPV genotypes were used, and allele calls were verified by visual inspection of individual cluster plots by using Evoker (www.sanger.ac.uk/science/tools/evoker). Population reference genotypes were only included for SI-RVPs with data available from >95% ExAC individuals. Association testing was performed by using one-tailed χ^2 statistics on collapsed SI-RPV data, comparing carriers and non-carriers in IBS patients compared with controls from ExAC.

Results

M-CAP/CADD combined analysis of all *SI* rare variants (N = 2146) resulted in the identification of 880 SI-RPVs with high predictive power (5% error rate for most variants). High-quality genotypes from IBS patients were available for 46 SI-RPVs, and 17 of these with at least 1 IBS carrier and ExAC reference data suitable for comparison were included in downstream association analyses (Table 1). We identified 88 IBS carriers (all single SI-RPV carriers; 3.99% of the entire cohort), with slightly higher prevalence in IBS-D (4.20%) and IBS-C (4.51%) than in other subtypes (Table 1). Compared with the large ethnically matched reference population from ExAC, most SI-RPVs occurred at higher frequency in IBS patients, and cumulative χ^2 tests (carriers of any SI-RPVs vs non-carriers) demonstrated significant associations and consistent effects on IBS risk (Table 1). In a simulation experiment, 1 million permutations of ExAC data resampled to match case sample size resulted >99% of the times in identical findings (SI-RPV carriers more common in IBS than in ExAC; $P < .001$).

Discussion

We provide further evidence linking rare functionally deleterious *SI* variations to IBS susceptibility. Although the large ExAC reference population (chosen to ensure genotype representation) does not include data on bowel symptoms, the observed association may represent an underestimation of the true genetic risk effects; the global prevalence of IBS is near 11%, and a significant proportion of ExAC individuals might thus be affected, with potential for inflating the background SI-RPV's carrier frequency among "controls" compared with an otherwise symptom-free reference group (type II error). The consistent observation of higher SI-RPV prevalence in IBS warrants further studies. This has the potential to identify groups among IBS patients for individualized management.

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Conflicts of interest
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