

Impaired cell surface expression and digestive function of sucrase-isomaltase gene variants are associated with reduced efficacy of low FODMAPs diet in patients with IBS-D

Recent seminal reports in *GUT*^{1,2} have proposed an association between gene variants of intestinal sucrase-isomaltase (SI) and the onset of irritable bowel syndrome (IBS).³ An increased risk of IBS-D (diarrhoea) symptoms in individuals harbouring the variant Val15Phe in the *SI* gene has been proposed due to partial trafficking impairment and reduced overall function of SI at the cell surface.^{1,2} These observations strongly suggested an association between Val15Phe and the onset of typical IBS-D symptoms (eg, diarrhoea and bloating⁴), which are similar to those in congenital SI deficiency.⁵ The presence of other SI gene variants (SIGVs) has been associated with poorer symptom response to a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols

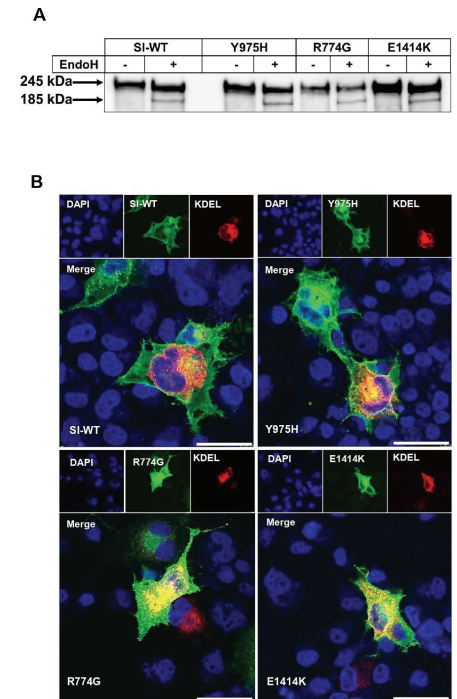


Figure 1 (A) Biosynthetic forms of SI wild type (SI-WT) and SI mutants. SI-WT or SI mutants were immunoprecipitated from transfected COS-1 cells, treated or not treated with Endoglycosidase H (Endo H) and subjected to Western blot analysis. 245 kDa is mature Endo H-resistant SI; 185 kDa is Endo H product of mannose-rich SI. (B) Cellular localisation of SI-WT and SI mutants in COS-1 cells. SI proteins were labeled with anti-SI antibodies (primary) and goat anti-mouse IgG (secondary) carrying DyLight 488 (green). KDEL-ds Red is the ER marker. DAPI labels the nucleus (blue). Bars=30µm.

(FODMAP) diet in IBS-D.² However, molecular, cellular and functional analyses of these variants are required to explain their potential effects on the digestive function of SI and diminished efficacy of the low FODMAP diet in reducing symptoms in patients with these variants. We therefore generated mutants of SI that harbour these SIGVs, namely R774G [rs147207752], Y975H [rs146785675] and E1414K [rs145734588]), by site-directed mutagenesis, expressed them in COS-1 cells and studied their biosynthetic protein forms, trafficking and function.

Wild-type SI is normally trafficked along the secretory pathway and reveals two biosynthetic forms, an endoplasmic reticulum (ER)-located mannose-rich form that matures in the Golgi to a complex glycosylated species and is trafficked with high sorting fidelity to the apical membrane.⁶ The trafficking competence of the three SI

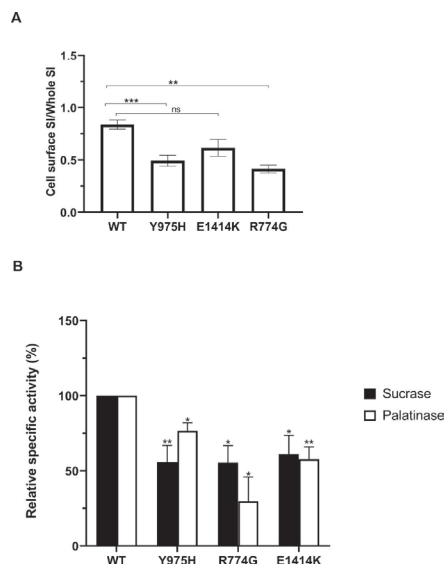


Figure 2 (A) Trafficking of SI proteins to the cell surface. SI-WT or SI mutants expressed in COS-1 cells were biotinylated and immunoprecipitated. Biotinylated cell surface SI was quantified versus total cellular SI. SI mutants at the cell surface were quantified versus SI-WT set to 100%. (B) Relative Specific enzyme activities of SI-WT and SI mutants. SI proteins were immunoprecipitated from transfected COS-1 cells and assayed for enzymatic activity versus sucrose or palatinose. Western blotting of similar samples was used to quantify the specific activities compared with SI-WT that was set to 100% for sucrase or palatinase. * $p < 0.05$, ** $p < 0.005$ and *** $p < 0.0005$.

mutants was assessed by endoglycosidase H (Endo H) that discriminates between the mannose-rich ER-located precursor and complex glycosylated mature forms.¹ Figure 1A shows that all three SIGVs are transport-competent, since both glycoforms were detected as assessed by Endo H treatment. These observations were supported by immunofluorescence images, which revealed these mutants in the ER colocalising with a ds-Red conjugated-protein carrying the ER retention signal, Lys Asp Glu Leu (KDEL) as well as in fluorescein isothiocyanate (FITC)-labelled punctate structures or lining the cell surface (figure 1B). Strikingly, substantial reductions in the cell surface localisation of the mutants were demonstrated in quantitative analyses using a biotinylation approach.¹ A decrease in about 50%–60% in the cell surface localisation was detected with SI-R774G and SI-Y975H being reduced significantly from wild type (figure 2A). Measurement of the specific enzymatic activities of the three mutants versus their protein expression levels in Western blots revealed a significant reduction of the

digestive capacity of the mutants towards sucrose and palatinose. The sucrase activity was significantly reduced between 40% and 45% and palatinase between 25% and 70% (figure 2B). Notably, the negative effects of the three SI mutants were substantially higher than those that have been demonstrated for the Val15Phe variant.^{1,2} Altogether, the reduced enzymatic activity together with the lower cell surface expression of the SI mutants would elicit substantial functional deficits in the digestive capacity of SI at the cell surface and provide a potential explanation for the efficacy of the low FODMAP diet in SIGVs carriers and emphasise a potential role of SI in the pathogenesis of IBS-D symptoms. Future studies should also focus on maltase-glucoamylase (MGAM) that specifically digests α -1,4 disaccharides and is together with SI a major α -glucosidase of the human small intestine. Here, the identification and phenotypic categorisation of prominent SI (and MGAM) mutations can facilitate diagnosis and early treatment.

In conclusion, the evaluation of low FODMAP diet efficacy in relation to SIGVs provides strong evidence that such a therapeutic approach is less effective in individuals with reduced SI activity.

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Correction notice This article has been corrected since it published Online First. The second author's name has been corrected.

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