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Impaired digestive function of sucrase-isomaltase in a complex with the Greenlandic sucrase-isomaltase variant

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ABSTRACT

Sucrase isomaltase (SI) is the most prominent disaccharidase in the small intestine. Congenital sucrase-isomaltase deficiency (CSID) is an autosomal recessive disorder caused by variants in the *SI* gene.

A homozygous frameshift mutation, c.273_274delAG (p.Gly92Leufs*8), has been identified in CSID in the Greenlandic population. This variant eliminates the luminal domain of SI and results in loss of its digestive function.

Surprisingly, the truncated mutant is transport-competent and localized at the cell surface; it interacts avidly with wild type SI and negatively impacts its enzymatic function.

The data propose that heterozygote carriers of p.Gly92Leufs*8 may also present with CSID symptoms.

Digestion of α-glycosidically-linked disaccharides occurs in the brush border membrane of enterocytes by sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM). SI is the most prominent disaccharidase in enterocytes by virtue of its broad glucosidase catalytic specificity towards α-1,2/ α-1,4/ α-1,6 glycosidic linkages [1]. SI is a type II membrane glycoprotein heavily N- and O-glycosylated and sorted to the apical membrane of enterocytes via O-glycans in the stalk region as well as interaction with lipid rafts [2]. Impaired trafficking of SI to the cell surface and reduced or absent enzymatic activity are hallmarks in the carbohydrate malabsorption disorder known as congenital sucrase-isomaltase deficiency (CSID) [3]. CSID is a rare disease elicited by hypomorphic variants in the coding region of the *SI* gene that can be homozygous or compound heterozygous [4]. CSID patients present with abdominal pain, flatulence, vomiting, cramps and osmotic diarrhea [5].

Whilst CSID is considered rare worldwide, it is highly prevalent in the Greenlandic population with almost 10 % of affected individuals [6]. CSID in the Greenlandic population results from a homozygous frameshift mutation in the SI coding sequence (c.273_274delAG; p.Gly92Leufs*8). This frameshift is located in close vicinity to the highly O-glycosylated stalk region [7] close to the transmembrane domain of SI (Fig. 1A). It is anticipated that this frameshift will result in truncation of both subunits, sucrase (SUC) and isomaltase (IM) and subsequent complete loss of SI digestive function. Nevertheless, unravelling the biochemical features and trafficking pattern of this SI mutant is essential towards understanding its potential interaction with wild-type SI (SI^{WT}) in heterozygote carriers of this mutation. Recently, in vitro studies suggested that heterozygote carriers of severe pathogenic SI variants may also present with CSID symptoms [8]. In fact, hetero-complexes comprising SI^{WT} and several SI mutants have been identified, in which the severity of SI mutants has negatively impacted the digestive function of the resulting hetero-complex as well as impairment of its trafficking to the cell surface.

Here, we studied the structural features and trafficking pattern of SI harboring the variant p.G92Lfs*8 and addressed its possible interaction with SI^{WT}, mimicking this situation in heterozygote carriers of p.G92Lfs*8.

We first generated a cDNA encoding a FLAG-tagged SI mutant harboring the p.G92Lfs*8 mutation ($SI^{G92Lfs*8}$) via site-directed mutagenesis from a pSG8-SI plasmid [3]. This clone was transfected into COS-1 cells, which were solubilized post-transfection and processed for immunoprecipitation with anti-FLAG antibodies followed by Western blotting [10]. Fig. 1B shows that $SI^{G92Lfs*8}$ was revealed at 27 kDa due to the truncation of SUC and IM. Control SI^{WT} appeared at 250 kDa, which is the expected size of the N- and O-glycosylated mature SI^{WT} [10]. As many other membrane proteins [11], SI^{WT} acquires mannose-rich N-linked oligosaccharides in the ER [3,12]. This type of glycosylation is sensitive towards endoglycosidase H (endo H). Similar to previous data [3] treatment of ST^{WT} with endo H resulted in two bands, an endo H-resistant protein corresponding to mature SI^{WT} and a 185 kDa polypeptide derived from the mannose-rich precursor (Fig. 1B). As expected,

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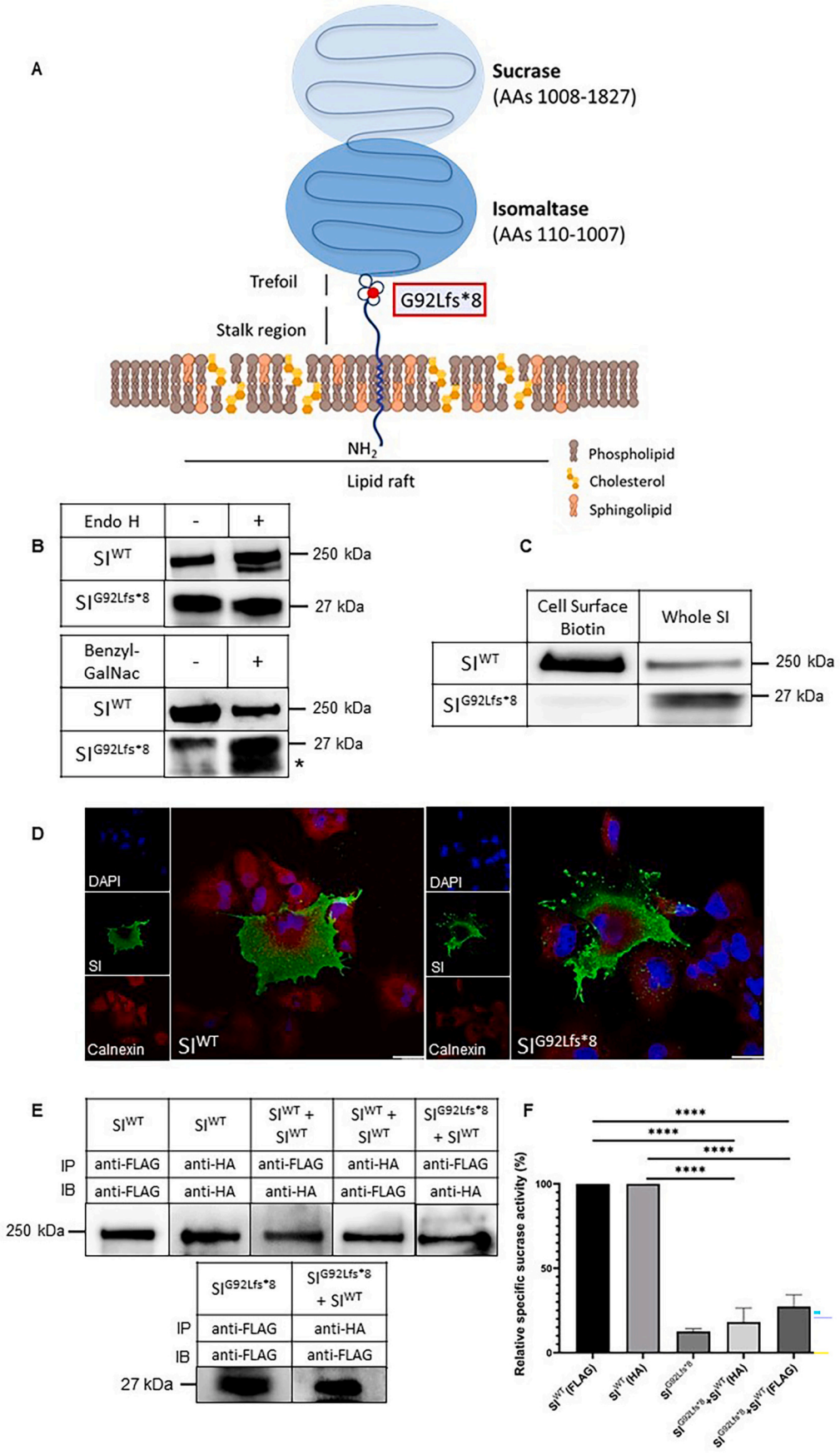

Fig. 1. Protein features and trafficking of $SI^{G92Lfs*8}$, interaction with SI^{WT} and enzymatic activity of the hetero-complex $SI^{G92Lfs*8}/SI^{WT}$.

$SI^{G92Lfs*8}$ was not cleaved with endo H, since all predicted N-glycosylation sites of SI are eliminated in truncated $SI^{G92Lfs*8}$. Unlike N-glycosylation sites, however, the heavily O-glycosylated Ser- and Thr-enriched stalk domain is retained in $SI^{G92Lfs*8}$ and can be O-glycosylated if the mutant has egressed the ER to the Golgi. One approach to determine whether $SI^{G92Lfs*8}$ exits the ER to the Golgi and is thus O-glycosylated is to treat cells expressing $SI^{G92Lfs*8}$ with benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (benzyl-GalNac), an inhibitor of O-glycosylation in the Golgi [2,13]. An inhibition of O-glycosylation would result in the appearance of a smaller protein band corresponding to the mutant devoid or partially devoid of O-glycans. In fact, Fig. 1B reveals two protein bands that were detected in the presence of benzyl-GalNac, one of which was markedly smaller than $SI^{G92Lfs*8}$ (referred to by the asterisk) indicative of O-glycosylation of the mutant in the Golgi apparatus. We further asked whether this mutant is trafficked beyond the Golgi to the cell surface and for this labelled cell surface of COS-1 cells expressing $SI^{G92Lfs*8}$ or SI^{WT} with biotin. Potentially cell surface located $SI^{G92Lfs*8}$ and the positive control SI^{WT} were immunoprecipitated with anti-FLAG antibodies and visualized with streptavidin. The results in Fig. 1C revealed a faint biotinylated $SI^{G92Lfs*8}$ band corresponding to the cell surface located form of this mutant. Expectedly, a substantially stronger biotinylated band corresponding to cell surface-located SI^{WT} was also detected, which is due to the higher number of cysteine residues in SI^{WT} (24 cysteines) as compared to only 3 cysteines in $SI^{G92Lfs*8}$ (Fig. 1C).

(A) Schematic presentation of sucrase-isomaltase showing the frameshift mutation (c.273_274delAG) at amino acid 92 (p.Gly92-Leufs*8) that results in truncation of the entire luminal domain comprising isomaltase and sucrase. The cartoon shows also the transmembrane domain that is associated with cholesterol- and sphingolipid-enriched membrane microdomains (lipid rafts), the O-glycosylated stalk region and the trefoil domain. (B) Expression and O-glycosylation of the $SI^{G92Lfs*8}$ mutant in COS-1 cells. FLAG-tagged forms of $SI^{G92Lfs*8}$ or SI^{WT}, as a control, were expressed in COS-1 cells and treated with or without endoglycosidase H (endo H) to assess the glycosylation pattern in the ER (upper panel). In another set of experiments COS-1 cells were treated with 2 mM of benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (Benzyl-GalNac) (lower panel). Samples were immunoprecipitated with anti-FLAG and immunoblotted with the same antibody. The upper panel reveals $SI^{G92Lfs*8}$ at an apparent molecular weight of 27 kDa that is endo H-resistant, unlike SI^{WT} that resolved into two protein bands concomitant with endo H-sensitivity of the mannose-rich SI^{WT} polypeptide (the lower band). The lower panel shows two bands corresponding to Benzyl-GalNAc-treated $SI^{G92Lfs*8}$, one of which is smaller than the original size of this mutant concomitant with inhibition of its O-glycosylation. (C) $SI^{G92Lfs*8}$ is localized at the cell surface. COS-1 cells transfected with SI^{WT} cDNA or $SI^{G92Lfs*8}$ cDNA were cell surface-biotinylated and the FLAG-tagged proteins immunoprecipitated with anti-FLAG antibodies and the cell surface proteins visualized by immunoblotting using streptavidin coupled to horseradish peroxidase. Faint, but definitive protein band corresponding to cell surface $SI^{G92Lfs*8}$ is shown. The protein band corresponding to SI^{WT} is several orders of magnitude stronger, due to the larger number of cysteines in SI^{WT} as compared to the mutant. (D) Immunofluorescence imaging reveals $SI^{G92Lfs*8}$ at the cell surface in transfected COS-1 cells. $SI^{G92Lfs*8}$ and SI^{WT} were expressed in COS-1 cells. The cells were permeabilized with saponin and processed for immunofluorescence as described before [9]. For detection of $SI^{G92Lfs*8}$ and SI^{WT} anti-FLAG antibody was the primary antibody and goat anti-mouse IgG carrying Alexa fluor 488 the secondary (green). For intracellular localization, rabbit antibodies against the ER marker calnexin was used as the primary antibody and goat anti-rabbit IgG carrying Alexa flour 568 as the secondary (red). DAPI was used to label the nucleus (blue). The fluorescence images were visualized in a Leica TCS SP5 confocal microscope with HCX PL APO lambda blue 40.0 × 1.25 OIL UV. (E) Interaction of co-expressed SI^{WT} and $SI^{G92Lfs*8}$ in COS-1 cells. FLAG-tagged $SI^{G92Lfs*8}$ was co-expressed with HA-tagged SI^{WT} and vice versa followed by reciprocal immunoprecipitations with anti-FLAG and Western blot with anti-HA or the other way around. (F) Relative specific sucrase (SUC) activities of SI^{WT} and $SI^{G92Lfs*8}$ expressed or co-expressed in COS-1 cells and immunoprecipitated with anti-FLAG or anti-HA antibodies. The immunoprecipitated proteins were assayed for enzymatic activity using sucrose as a substrate and GOD-PAP (Axiom Diagnostics, Florida, United States) as a glucose oxidation detection solution that was measured at 492 nm. Similar samples were analyzed by Western blotting and the protein band intensity was used to quantify the specific activities of the $SI^{G92Lfs*8}$ and SI^{WT} proteins. Relative specific activities are measured in comparison to SI^{WT} that was set to 100 % for sucrase. (Paired *t*-test, **** $p < 0.00005$, S.E.M., $n = 3$).

The biotinylation data were substantiated by an immunofluorescence imaging approach as previously described [9]. Transfected COS-1 cells were permeabilized and stained with anti-FLAG recognizing the tagged forms of $SI^{G92Lfs*8}$and SI^{WT}, anti-calnexin for ER staining and DAPI for nucleus staining. Fig. 1D shows prominent fluorescence labeling of $SI^{G92Lfs*8}$ at the cell surface and little co-localization of the mutant with calnexin in the ER. Expectedly, SI^{WT} was found at the cell surface. The biochemical and immunofluorescence data indicate that $SI^{G92Lfs*8}$ is transport-competent along the secretory pathway to the cell surface.

This raises the question of whether an interaction between $SI^{G92Lfs*8}$ and SI^{WT} takes place along the secretory pathway and what impact the loss-of-function $SI^{G92Lfs*8}$ mutant may have on the overall SI digestive function. We therefore co-expressed FLAG-tagged $SI^{G92Lfs*8}$ and HA-tagged SI^{WT} in COS-1 cells mimicking thus the situation in heterozygote carriers, examined their potential interaction and enzymatic function. Potential hetero-complexes were immunoprecipitated with antibodies against one tag and visualized by Western blotting with antibodies against the other tag. Fig. 1E shows that both SI forms interacted with each other, since $SI^{G92Lfs*8}$ was pulled down with the antibody that immunoprecipitated SI^{WT} (lower panel) or vice versa (upper panel). Finally, the activity of the $SI^{G92Lfs*8}/SI^{WT}$ hetero-complex towards sucrose was measured and revealed a drastic reduction of >65 % as compared to that of SI^{WT}. Expectedly the activity of the mutant per se was below detection limit (Fig. 1F).

In conclusion, the study shows that the most common mutation in CSID in the Greenlandic ethnic group, p.G92Lfs*8, results in a truncation of SI and elimination of its two catalytic subunits, SUC and IM, resulting in a functionally inactive enzyme in homozygotes. Remarkably, the truncation of almost the entire luminal domain does not affect the trafficking of the remaining short protein segment, comprising the O-glycosylated stalk and transmembrane domains, to the cell surface. Previous studies with successive truncations of the Ser/Thr residues of the stalk region revealed that this stalk region is essential for an efficient sorting of SI to the apical membrane [2]. The current work substantiates these data and provides a direct evidence with a naturally-occurring mutant in CSID that only the stalk region together with transmembrane domain are necessary and sufficient for transport-competence of SI.

The high prevalence of the p.Gly92Leufs*8 variant in the Greenlandic population (about 10 % homozygotes [7]) predicts also a high frequency of this mutation in heterozygotes (around 43 % heterozygotes according to the Weinberg Eq. [14]) and raises the question of whether heterozygotes may be also affected in their digestive capacity towards sucrose (or other carbohydrates) and present with symptoms of CSID. While relevant clinical data are not available, our work shows that an interaction between SI^{WT} and $SI^{G92Lfs*8}$ takes place resulting in a substantial reduction of the enzymatic activity of SUC (>65 %). Recent observations unravelled the existence of hetero-complexes of SI^{WT} with pathogenic variants of SI [8]. These complexes are impacted by the severe pathogenicity of the SI mutants, such as those harboring the p.V577G, G1073D and p.F1745C variants resulting in retention of these hetero-complexes in the ER along with a drastic reduction of their SUC

activity [8]. A similar retention of the $SI^{G92Lfs*8}/SI^{WT}$ complex in the ER with severe impact on the digestive capacity of the hetero-complex is unlikely to take place, since $SI^{G92Lfs*8}$ per se as well as SI^{WT} are efficiently trafficked to the cell surface. Moreover, the hetero-complex $SI^{G92Lfs*8}/SI^{WT}$ retained partial digestive function (<35 %) that may partially compensate for the inactive $SI^{G92Lfs*8}$ and reduce the severity of typical CSID symptoms in heterozygote carriers of this variant as compared to homozygotes. The loss of SI function due to p.G92Lfs*8 appears to positively impact the metabolic health of Greenlandic cohorts due to increased levels of acetate [15]. Moreover, analysis of the body composition and lipid profiles did not reveal significant variations between homozygote and heterozygote carries. Nevertheless, the enzymatic activities of SI in these cohorts were not assessed [15]. Therefore, it is important to investigate the impact of p.G92Lfs*8 on the digestive function of SI and intestinal physiology in heterozygote carriers in view of its high occurrence in the Greenlandic population.

Author contributions

Conceptualization, H.Y·N; Methodology, H.Y·N.; Software, S.T.; Data Curation, S.T.; Visualization, S.T. and H.Y·N.; Formal Analysis, S.T. and H.Y.N; Supervision, H.Y.N.; Writing—Original Draft Preparation, S.T.; Writing—Review and Editing, H.Y.N; Funding Acquisition, H.Y.N. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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