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Investigating intestinal epithelium metabolic dysfunction in celiac disease using personalized genome-scale models

Chloe V. McCreery[1,2,3,4], Drew Alessi[2,5†], Katarina Mollo[6†], Alessio Fasano[2,3,6] and Ali R. Zomorrodi[2,3,6*]

Abstract

Background Celiac disease (CeD) is an autoimmune condition characterized by an aberrant immune response triggered by the ingestion of gluten, which damages epithelial cells lining the small intestine. Small intestinal epithelial cells (sIECs) play key roles in the enzymatic digestion and absorption of nutrients, maintaining gut barrier integrity, and regulating immune response. Chronic inflammation and tissue damage associated with CeD disrupt the intricate network of metabolic processes in sIECs that support these functions, impairing their ability to perform their essential roles. However, the specific disrupted metabolic processes underlying sIECs dysfunction in CeD remain largely undefined.

Methods To address this knowledge gap, personalized, sex-specific genome-scale models of sIECs metabolism were constructed using transcriptional data from intestinal biopsies of 42 subjects with active CeD, CeD in remission (on a gluten-free diet), and non-CeD controls. These models were computationally simulated under relevant dietary conditions for each group of subjects to assess the activity of several metabolic tasks essential for sIECs function and to profile metabolite secretion into the bloodstream and intestinal lumen.

Results Significant alterations in the activity of 28 essential metabolic tasks were observed in active CeD and remission CeD, impacting critical processes integral to sIECs function such as oxidative stress regulation, nucleotide synthesis and DNA repair, energy production, and polyamine and amino acid metabolism. Additionally, altered secretion profiles of several metabolites, encompassing amino acids, vitamins, polyamines, lipids, and fatty acids, into the bloodstream were detected in active CeD and remission CeD patients. These findings were partially supported by comparisons with independent external datasets and further corroborated through extensive review of existing literature. Furthermore, a drug target analysis was performed, identifying 22 FDA-approved drugs that target genes encoding impaired sIECs metabolic functions in CeD, potentially helping to restore their normal activity.

Conclusions This study unveils new insights into the metabolic reprogramming of sIECs in CeD, highlighting specific dysregulated metabolic processes that compromise cellular functions essential for gut health. These findings offer a foundation for developing therapeutic interventions targeting impaired metabolic processes in CeD.

Keywords Celiac disease, Small intestinal epithelial cells, Metabolism, Genome-scale modeling

†Drew Alessi and Katarina Mollo are joint second authors.

*Correspondence:
Ali R. Zomorrodi
azomorrodi@mgh.harvard.edu
Full list of author information is available at the end of the article

Background

The intestinal epithelium is a monolayer of columnar epithelial cells that plays a pivotal role in maintaining the integrity and functionality of the gastrointestinal tract. These cells serve as a critical barrier between the luminal contents of the intestine and the underlying tissues, selectively preventing the permeation of luminal endotoxins, pathogenic microorganisms, and other antigens while allowing the absorption of nutrients, useful microbial products, electrolytes, and water. Additionally, intestinal epithelial cells contribute to mucosal immune regulation and immune tolerance to dietary compounds. However, in the case of inflammatory diseases, the function of intestinal epithelial cells can be profoundly compromised, allowing the dysregulated passage of endotoxins, pathogens, antigens, and other pro-inflammatory substances into the human body. This can lead to an overactive immune response and subsequently inflammation and disease. One example of such inflammatory conditions is celiac disease (CeD), which is the focus of this study.

CeD is an autoimmune disease triggered by the consumption of gluten, a composite protein found in wheat, rye, barley, and other cereal grains. Currently, this disease is estimated to affect over three million Americans and 1.4% of the global population, with a slightly higher prevalence in women [1, 2]. CeD has a strong genetic basis with individuals carrying certain human leukocyte antigen (HLA) alleles, namely HLA-DQ2 and HLA-DQ8, being at significantly elevated risk of developing the disease. Chronic exposure to gluten in individuals with genetic predisposition for CeD elicits an abnormal immune response to undigested gluten peptides, particularly those from gliadin, a component of gluten which is resistant to proteolytic digestion. Although CeD and its symptoms can be managed by following a strict gluten-free diet, no treatments or drugs currently exist to complement this diet [2, 3].

Small intestinal epithelial cells (sIECs) are also major sources of immunomodulatory factors in the intestinal micromilieu through which they interact with and profoundly influence the immune response to gluten. For example, we showed in a previous study that the macrophage response to gliadin is influenced by sIECs [4]. Additionally, evidence suggests that sIECs may directly contribute to the inflammatory cascade that characterizes CeD through the production of pro-inflammatory cytokines in the presence of gliadin [2]. It is also well known that active CeD causes poorer absorption of nutrients due to chronic inflammation and intestinal damage [5, 6]. Several studies, including one from our team [7], have investigated alterations in sIECs gene expression levels both in active CeD [7–10] and remission CeD [7, 8, 11], which have significantly enhanced our understanding of biological processes associated with intestinal damage in CeD.

The critical functions of sIECs rely on a complex, finely tuned network of metabolic processes that support energy production, cell growth, and synthesis of essential bioactive compounds. Chronic inflammation and tissue destruction in CeD disrupt these metabolic networks in sIECs. These metabolic disruptions significantly compromise several aspects of gut health relevant to CeD such as nutrient absorption and digestion, gut barrier integrity, immune cell interactions, and the overall inflammatory response to gluten. However, critical gaps remain in current knowledge regarding the specific impaired metabolic processes that underlie sIECs dysfunction in CeD. Addressing this knowledge gap could help unravel how the interplay between gene expression, metabolism, immunity, and diet may contribute to CeD pathogenesis.

GEnome-scale Models (GEMs) of metabolism are an ideal tool for studying sIECs metabolism. These models encapsulate all metabolic reactions encoded by the genome of an organism and can be constructed from sequenced and annotated genomes. For human cells, one can use transcriptional profiles from specific cell types or tissues to infer enzymes or reactions that are active and construct cell- or tissue-specific GEMs. The reconstructed GEMs can then be computationally simulated using Constraint-Based Reconstruction and Analysis (COBRA) methods to predict various system-level metabolic properties such as the growth capacity, nutrient absorption, metabolite secretion, and internal reaction fluxes [12]. GEMs have demonstrated significant promise in modeling human metabolism, disease-associated metabolic alterations, and drug target identification [13–18].

In this study, we aimed to investigate the metabolic landscape of sIECs in CeD by utilizing GEMs of metabolism. To this end, we constructed patient-specific GEMs of sIECs metabolism for individuals with active CeD, CeD in remission, and non-CeD controls using transcriptional data from intestinal biopsies of these subjects. Utilizing these GEMs, we gained a deeper understanding of metabolic alterations in sIECs associated with CeD. Several essential metabolic tasks were identified to have altered activity in both active CeD and remission CeD compared to non-CeD controls. Additionally, altered sIECs secretion profiles of several metabolites into the bloodstream were identified across the study groups. We further utilized the identified differentially active metabolic tasks to propose drugs that have the potential to bring back these altered metabolic functionalities to normal levels in CeD patients.

Methods

Personalized sIECs GEM construction and analysis

An existing literature curated GEM of metabolism for sIECs was used as a baseline model [19]. This GEM contains 1282 reactions and 433 unique metabolites located in five intracellular compartments (cytosol, nucleus, mitochondria, peroxisome, and endoplasmic reticulum) and two extracellular compartments representing the intestinal lumen and the bloodstream to reflect the apical and basolateral sides of the sIECs. Personalized computational sIECs models were then constructed from this baseline model by incorporating gene expression (RNA sequencing) data collected from the duodenal biopsies of subjects with active CeD, CeD in remission, and healthy controls [7] into Flux Balance Analysis (FBA) models. This was done by constraining the reaction fluxes in GEMs according to gene expression data for each subject (McCreery and Zomorrodi, manuscript under preparation). To create these personalized sIECs GEMs, genes were grouped into 20 clusters based on their level of expression across all samples using the StanDep pipeline [20], implemented in MATLAB 2018. Within each of these clusters, gene expression levels were normalized to ensure uniformity between samples within that cluster. This clustering strategy allows for the retention of genes with low expression levels and safeguards against their removal. Normalized gene expression data were used to impose soft constraints on the lower and upper bound on reaction fluxes in the network. The weighted sum of a key objective and the deviations from the imposed bounds on reactions in the GEM (i.e., deviations from gene expression data) was used as the objective function. In this study, the flux of reactions representing essential metabolic tasks or exchange reactions for metabolites that can be secreted into the bloodstream or feces was maximized as the key objective, while deviations from the imposed reaction bounds were minimized.

In silico diets

An average American diet was obtained from food intake data recorded by the 2007–2008 National Center for Health Statistics' National Health and Nutrition Examination Survey [21] (Additional File 1). From this survey, a diet was reconstructed by calculating the average intake of each item listed in the survey's 226 food and drink groups. Similarly, an average gluten-free diet was created from a list of 21 food items within a typical gluten-free diet reported by CeD patients at Massachusetts General Hospital, Boston, MA, USA (Additional File 1). This list of food items from each diet was then converted to bounds on uptake fluxes of 85 and 89 metabolites, for the Average American and gluten-free diets, respectively, using the Diet Designer tool in the Virtual Metabolic Human (VMH) database [22]. Aside from these nutrients acquired from the diet, the uptake of oxygen, bicarbonate, and asparagine from the blood compartment, as well as bicarbonate from the lumen compartment, were allowed for both diets as proposed in [19]. This ensures flux consistency in the models for both the Average American and gluten-free diets. For European cohorts in the validation dataset, we utilized the pre-designed in silico Average European diet and a European gluten-free diet available in the VMH Additional File 1). To computationally simulate a diet, the lower bound for each metabolite's exchange reaction was set to the respective value in the in silico diet formulation to reflect its uptake limit.

To incorporate sex as a variable in our analyses, each sIECs GEM's biomass reaction was constrained between a specific lower and upper bound that were sex-specific. To calculate the sex-specific bounds, the organ-resolved male and female whole-body model (WBM) of metabolism developed by Thiele et al. [23] were utilized. In addition to the organ-specific maintenance biomass reactions, these WBMs contain a whole-body biomass reaction that is constrained to carry a flux of 1 mmol/day/person in FBA simulations. Here, these male and female WBMs were simulated under the Average American and Gluten-free diets and determined the maximum flux of the sIECs maintenance biomass reaction in the WBMs with or without constraining the whole-body biomass reaction flux. The relative percentage of the sIECs maintenance biomass reaction flux when constraining the whole-body biomass reaction flux to 1 mmol/day/person compared to that without was then calculated, i.e., 100 × (sIECs maintenance biomass reaction flux in the WBM with the whole-body biomass reaction flux constrained at 1)/(sIECs maintenance biomass reaction flux in the WBM without constraining the whole-body biomass reaction flux). Similar calculations were performed when minimizing the sIECs maintenance biomass reaction within the WBMs, but the minimum values turned out to be the same as maximum values. These percentages were calculated to properly constrain the biomass reaction flux in sIECs GEMs for this study. To this end, the maximum biomass reaction flux for sIECs GEMs constrained by transcriptional data under each diet was calculated first. Next, the percentage obtained from the male or female WBM was multiplied by each IEC GEM's maximum biomass flux under their respective diet, and the resulting value was set as the model's biomass reaction flux. A similar approach was used for European cohorts in the validation dataset. Calculating biomass flux separately for male and female samples allowed us to seamlessly incorporate sex as a variable in our analyses.

Computational simulation of GEMs was then performed using the COBRA Toolbox [12] in Python 3.8. For

each metabolic task tested, its respective reaction flux was maximized while also minimizing deviations from gene expression data imposed using the soft constraints as noted above (McCreery and Zomorrodi, manuscript under preparation).

Shadow price analysis

Shadow price analysis was performed using the COBRA Toolbox in Python 3.8. After performing FBA for each metabolic task, the total number of metabolites with non-zero shadow prices were recorded.

Principal component analysis (PCA)

The optimal fluxes of essential metabolic tasks were used as features for PCA (59 features), while the subjects served as samples (42 samples). PCA was performed using the *scikit-learn* (*sklearn*) package in Python.

External validation datasets

Three independent studies providing whole-genome transcriptional data from intestinal biopsies of subjects with active CeD, remission CeD, and healthy controls were utilized to create a multi-cohort validation dataset. These included RNA-seq data from 44 healthy controls and 51 active CeD patients reported by Abadie et al. [9], RNA-seq data from 20 active CeD patients and 19 controls presented by Bragde et al. [10], and RNA-seq data from 8 remission CeD patients (on a strict gluten-free diet) and 3 controls available from Dotsenko et al. [11].

Batch removal

Batch effects were removed from gene expression data for the cohorts in the validation dataset by using the ComBat-seq pipeline [24]. This analysis was conducted in Python with the *pycombat_seq* function from the *inmoose* 0.7.2 library and the *pycombat* function from the *pycombat* 0.20 library.

Evaluating concordance of results with the validation dataset

We calculated the directional concordance rate (DCR) based on the direction of change (increase or decrease) for the essential metabolic tasks showing significant altered activity between study groups (Mann–Whitney *U*, $q < 0.05$) and the same tasks in the validation dataset. The DCR was calculated as $100 \times$(number of metabolic tasks with concordant direction of change between datasets)/(total number of tasks compared).

Statistical analyses

Statistical hypothesis testing was based on the Mann–Whitney *U* (Wilcoxon rank sum) test implemented using the *wilcox* function in R (with the *exact* argument set to False). All raw *p*-values were adjusted for multiple testing when relevant based on the Benjamini-Hochberg (BH aka FDR) method using the *p.adjust* function in R. Statistical significance was determined based on an adjusted *p*-value (*q*-value) threshold of 0.05.

Drug target analysis

Genes corresponding to metabolic tasks with significant differential activity were extracted from the reactions' gene-protein-reaction (GPR) rules in sIECs GEMs. These genes were then searched within the DrugBank database [25] to identify drugs that can up- or down-regulate their expression. Flux coupling analysis was performed using Flux Coupling Finder 2 (FCF2) v0.95b in MATLAB 2023b [26, 27].

Results

Reconstruction of personalized sIECs GEMs

Patient-specific GEMs of sIECs metabolism were created computationally using a previously published sIECs GEM [19] as a base model and patient-derived transcriptional data [7]. This base sIECs GEM was constructed based on extensive literature reviews and manual curations [19]. Whole-genome RNA sequencing data collected in a previous study from our team [7], from duodenal biopsies of 42 subjects including 12 patients with active CeD, 15 with CeD in remission (on a gluten-free diet), and 12 healthy (non-CeD) controls were utilized to construct personalized GEMs. To computationally tailor the GEM for each individual in the cohort, each patient's transcriptional profile was incorporated into a constraint-based model of the base sIECs GEM (Fig. 1A). Constraint-based methods enable the computation of reaction fluxes within a GEM and the prediction of systems-level metabolic properties, offering insights into the complex interplay between numerous metabolic reactions and pathways in sIECs at a genome-wide scale.

The personalization of the constraint-based model for each GEM was achieved by constraining metabolic reaction fluxes in the base sIECs GEM according to gene expression levels for each individual. To further personalize these computational models beyond integration with patient-derived gene expression data, sex-specific sIECs growth rates for each individual were incorporated into our analysis using values obtained from the male and female Whole-Body Models of metabolism developed previously [23] (see the "Methods" section).

The resulting 42 computational GEMs thus represent the personalized metabolic landscape of sIECs in active CeD, remission CeD, and non-CeD individuals. Each GEM contains two extracellular compartments representing the apical side (intestinal lumen) and the basolateral side (arterial blood) (Fig. 1B). This allows for the

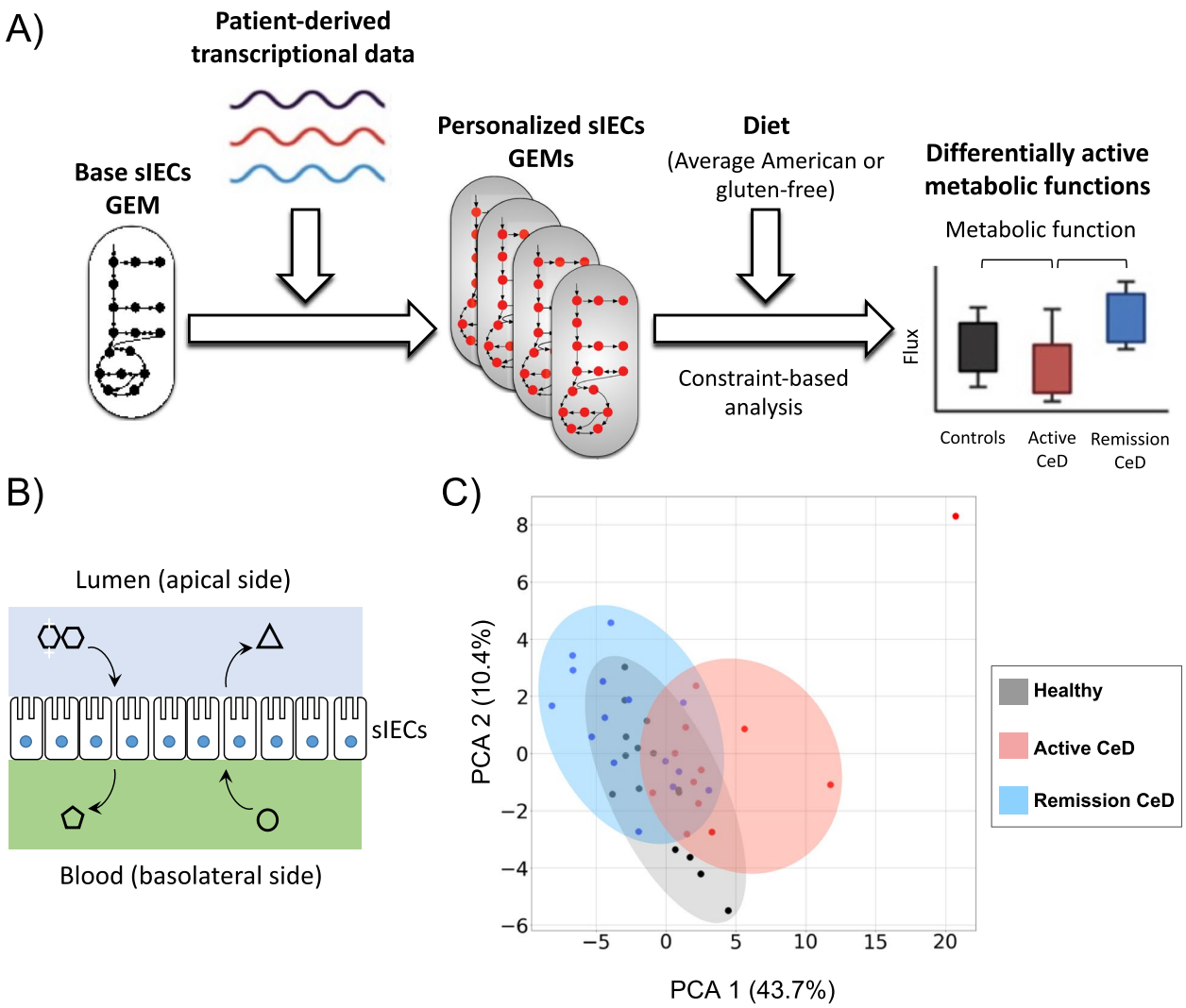

Fig. 1 Personalized modeling and analysis of sIECs metabolism in CeD using genome-scale models. **A** Overview of the workflow for the construction and simulation of patient-specific sIECs GEMs using patient-derived transcriptional data. **B** Each sIECs GEM includes two extracellular compartments representing the basolateral and apical sides. **C** Principal Component Analysis (PCA) of sIECs metabolism using the 59 essential metabolic tasks as features

modeling of nutrient absorption from the lumen (apical uptake from dietary inputs) and from arterial blood (basolateral uptake), as well as metabolite secretions into the lumen (feces) and blood [19].

Computational investigation of sIECs metabolism using personalized GEMs

The personalized sIECs GEMs were simulated under a relevant diet for each group of subjects. An average American diet was formulated and used for the active CeD patients and controls, and a gluten-free diet was designed based on the food items within a typical gluten-free diet reported by CeD patients at the recruitment site for this cohort and utilized for those with CeD in remission. The nutritional profiles of these two diets exhibit differences in their macronutrient and micronutrient compositions. In particular, the Average American diet is characterized by a higher proportion of lipids and lower quantities of carbohydrates and proteins. The detailed macronutrient and micronutrient composition of these diets is provided in Additional File 1.

To elucidate metabolic alterations in sIECs associated with CeD, the capability of sIECs in these subjects to perform 59 defined metabolic tasks essential for their growth and functionality, curated previously by Sahoo et al. [19], was assessed computationally (Additional File 1). These tasks encompass a wide range of essential functions examples of which include the synthesis of amino

acids and nucleotides, ATP generation via the TCA cycle, the urea cycle, heme synthesis and degradation, and glucose metabolism. Given the fundamental nature of these tasks for sIECs operation and function, they should all be active in a healthy sIECs GEM [19]. In this analysis, the flux through metabolic reactions in GEMs representing each task's activity was maximized. The potential of sIECs in secreting metabolites into the bloodstream and intestinal lumen was also investigated by maximizing the flux of exchange reactions corresponding to each metabolite. The predicted activity of each metabolic task and the secretion level of each metabolite was recorded and compared between each pair of study groups.

Overall metabolic landscape of sIECs with respect to essential metabolic tasks across conditions

To gain insights into how the overall metabolic profiles of sIECs with respect to the essential metabolic tasks might be different across the three study groups, principal component analysis (PCA) was employed. This analysis shows a partial stratification among the three study groups based on the metabolic profiles of sIECs, as captured by the essential metabolic tasks (Fig. 1C). Subjects in the remission CeD group tend to cluster on the left along the first principal component (PC1), which explains 43.7% of the variance, while subjects with active CeD cluster more toward the right along PC1. This separation implies a divergence in the metabolic capabilities of these two groups. Healthy subjects occupy an intermediate position between the remission CeD and active CeD along PC1. The healthy group shows noticeable overlap with the remission CeD group, demonstrating shared metabolic profiles, and less overlap with the active CeD group, suggesting a metabolic shift in the active CeD group. Less distinct separation between the groups is observed along PC2, which accounts for an additional 10.4% of variance.

Of note, an individual from the active CeD group is distinctly positioned away from the main cluster. Clinical metadata review revealed no anomalies for this subject. Given the lack of any other identifiable errors in the collection or handling of biospecimens or data collection for this participant and considering the potential biological relevance of the atypical metabolic profile observed, we opted to retain this subject in all subsequent analyses. A sensitivity analysis to evaluate the impact of excluding this subject from further analyses showed minimal impact on the results, highlighting the robustness of our findings (see Additional File 2).

Essential metabolic tasks exhibiting significant differential activity across conditions

By investigating the activity of the 59 metabolic tasks essential for the functionality of sIECs, a total of 28 unique metabolic tasks were identified to have significant differential activity between at least one pair of the study groups (Mann–Whitney U, $q < 0.05$) (Fig. 2, Additional File 1). These include 17 tasks showing significant differential activity between the active CeD patients and healthy controls, 27 tasks between the active CeD and remission CeD groups, and six tasks between remission CeD and healthy controls. These metabolic tasks play pivotal roles in maintaining the structural and functional integrity of small intestine, and their altered activity impacts various aspects of gut health such as gut barrier function, inflammatory response, and oxidative stress regulation. In the subsequent sections, we explore these metabolic tasks in greater detail.

Altered mitochondrial metabolism in active CeD

Six metabolic tasks that exhibited differential activity between conditions were directly or indirectly related to the TCA (tricarboxylic or citric acid) cycle, which is responsible for deriving energy from nutrients in in the mitochondria. These tasks include mitochondrial aspartate transaminase, mitochondrial malic enzyme, malate dehydrogenase, citrate synthase, riboflavin kinase, and L-lactate secretion all of which showing elevated flux in the active CeD group relative to one or both other groups (Fig. 2; also see Additional File 2 [28] for more details about these tasks). Notably, no genes corresponding to these metabolic tasks—*GOTL1* for aspartate transaminase, *CS for* citrate synthase, *ME2*/*ME3* for malic enzyme, and *MDH/MDH1B* for malate dehydrogenase—show significant differences in expression levels among the groups (Mann Whitney U, $q < 0.05$; Additional File 3: Fig. S1).

Differentially active metabolic tasks involved in reactive oxygen species (ROS) synthesis and metabolism

Six differentially active metabolic tasks contribute directly or indirectly to ROS production or metabolism in the gut. Of these, nitric oxide synthase, xanthine oxidase, argininosuccinate lyase engage in ROS synthesis while superoxide dismutase, transketolase 2, and desmosterol reductase are involved in ROS breakdown and regulation. All these metabolic tasks exhibit elevated activity in active CeD compared to the remission CeD and/or control groups (Fig. 2). Again, no significant changes in the expression levels of the genes producing these enzymes—*SOD2* for superoxide dismutase, *XDH* for xanthine oxidase, TKT, TKTL1, and *TKTL2* for transketolase, *ASL* for argininosuccinate lyase, and *NOS1*/*NOS2*/*NOS3* for nitric oxide synthase—were observed (Additional File 3: Fig. S1).

Nitric oxide synthase produces nitric oxide (NO), a compound which plays an important role in regulating

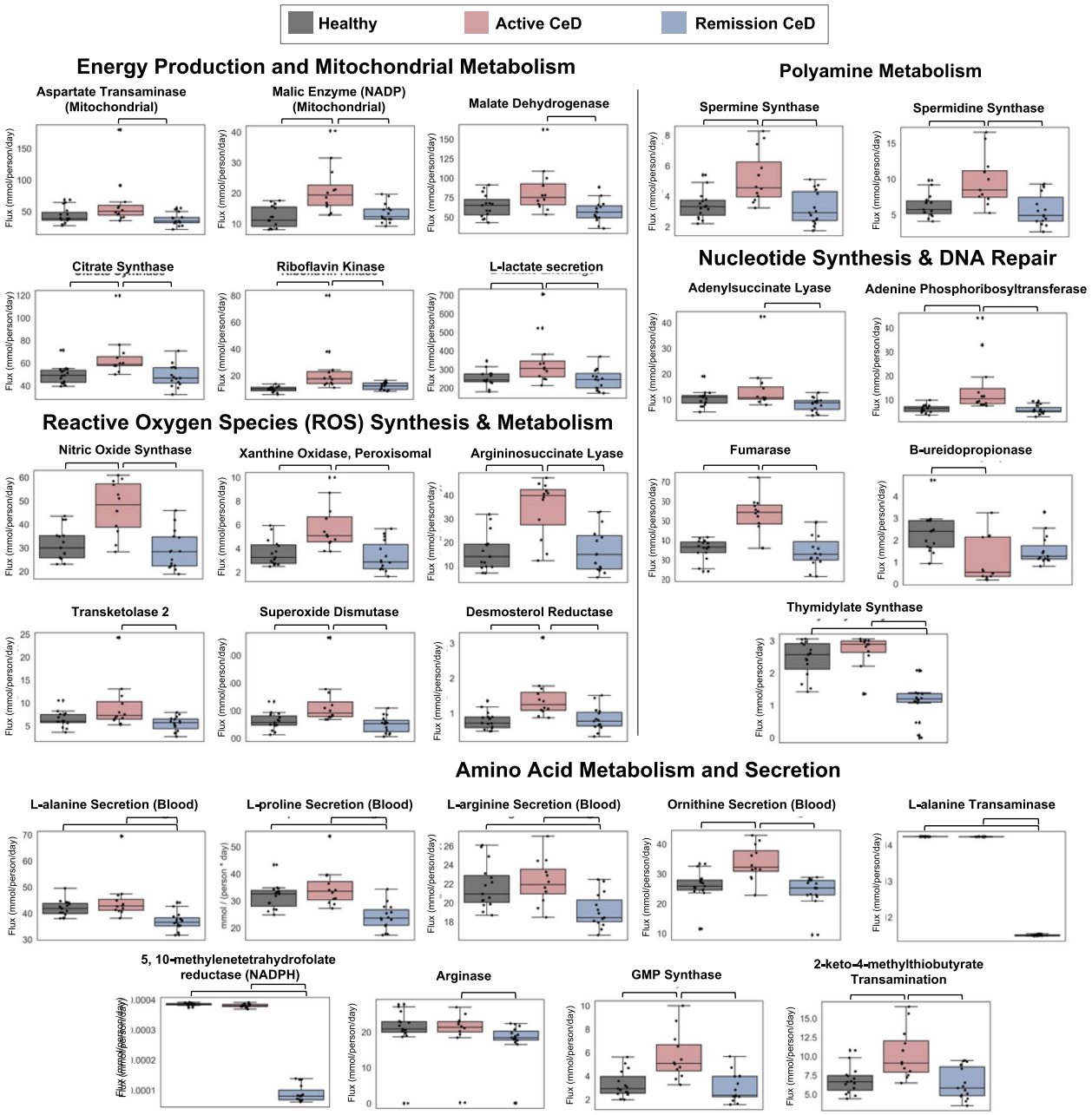

Fig. 2 Significantly altered metabolic tasks in sIECs across study groups. Essential metabolic tasks that exhibit significant differential activity between active CeD, remission CeD, and non-CeD controls (Mann–Whitney *U*, $q < 0.05$), impacting various aspects of small intestine metabolism and function. The full list of metabolic tasks analyzed along with the adjusted *p*-values (*q*-values) is provided in Additional File 1

and maintaining intestinal barrier integrity; however, it can also cause oxidative stress and damage, if present at high concentrations [29]. Xanthine oxidase is also involved in production of hydrogen peroxide, which is another ROS. Argininosuccinate lyase catalyzes the cleavage of argininosuccinic acid to generate arginine, a substrate for nitric oxide synthase.

Of the metabolic tasks involved in ROS breakdown, superoxide dismutase is a main ROS-scavenging enzyme that reduces superoxide radicals, thereby helping to decrease oxidative stress [30]. Transketolase 2 has been also shown to minimize ROS infiltration into the intestine and support intestinal barrier maintenance in animal models of IBD [31]. Finally, desmosterol reductase

is a key enzyme in the cholesterol synthesis pathway [32] that has cytoprotective effects, preventing apoptosis and improving cell survival through the mitigation of ROS production [33].

Elevated polyamine synthesis in CeD

Spermine synthase and spermidine synthase, two enzymes responsible for spermine and spermidine synthesis, showed significantly higher activity in active CeD patients compared to both healthy controls and remission CeD subjects (Fig. 2). Spermine, the product of spermine synthase, is a polyamine that promotes cellular growth, modulates gut epithelium integrity, and supports healthy epithelial barrier function [34]. Spermidine, the product of spermidine synthase, is a precursor for spermine biosynthesis.

Differentially active metabolic tasks involved in nucleotide synthesis and DNA repair

Five functions showing significant variations in activity across the study groups are implicated in nucleotide synthesis and DNA repair essential for sIECs growth and regeneration. Of these, adenylosuccinate lyase, adenine phosphoribosyltransferase, and fumarase show significantly higher activity in active CeD, while B-ureidopropionase exhibits diminished activity in active CeD compared to controls, and thymidylate synthase exhibits less activity in remission CeD compared to active CeD and controls. Additional details about these tasks are provided in Additional File 2 [35–38].

Amino acid production and secretion is altered in both active and remission CeD patients

Significant altered activity was observed in nine metabolic tasks related to amino acid production, metabolism, and transport in the active and remission CeD groups. Four of these tasks engaged in the secretion of amino acids into the blood. Specifically, L-alanine, L-arginine, and L-proline exhibited significantly reduced secretion flux into the blood in remission CeD, while ornithine showed a significant increased secretion in the active CeD group (Fig. 2).

The other five tasks are related to amino acid synthesis and metabolism. Of these L-alanine transaminase, 5, 10-methylenetetrahydrofolate reductase (NADPH), and arginase show significantly reduced activity in remission CeD (see Additional File 2 [39] for more details about these functions). The remaining tasks are GMP synthase and 2-keto-4-methylthiobutyrate transamination, which are related to L-glutamate production, both of which showing elevated fluxes in active CeD.

Metabolites differentially secreted into the bloodstream and intestinal lumen

To investigate the capacity of sIECs in secreting metabolites into the bloodstream and intestinal lumen across conditions, the maximum secretion potential of various metabolites was quantified. These metabolites correspond to 166 exchange reactions within the sIECs GEMs, which transport metabolites from the cytosol into either the bloodstream or lumen (Fig. 1B). Through this analysis, 58 distinct metabolites (other than those discussed above as essential metabolic tasks) exhibiting significant variations in secretion flux into the blood or lumen between at least two conditions were identified (Mann–Whitney *U*, $q < 0.05$, Fig. 3). Of these, 57 showed differential secretion into the bloodstream (11 between active CeD and controls, 56 between active CeD and remission CeD, and 47 between remission CeD and controls). Also, two metabolites showed differential secretion into the lumen (both between active CeD and the other two groups). These metabolites span various categories such as essential and non-essential amino acids, vitamins, lipids and fatty acids, polyamines, and nucleosides/nucleotides.

Among the metabolites exhibiting elevated secretion in active CeD relative to remission CeD and controls are polyamines (putrescine, spermidine, and spermine) as well as metabolites involved in oxidative stress pathways (nitric oxide and urate). Nitric oxide secretion into the lumen and urate secretion into the blood were elevated in active CeD compared to both healthy controls and remission CeD. Conversely, we observed the reduced secretion of certain vitamins and essential nutrients such as (R)-pantothenate (vitamin B_5), vitamin D_3, and choline, in active CeD relative to remission CeD.

The remission CeD group also shows distinct patterns in metabolite secretion into the blood. Specifically, remission CeD patients displayed elevated levels of essential fatty acids and vitamins, such as linoleic acid, alpha-linolenic acid, and phosphatidylcholine, as well as vitamins like phylloquinone (vitamin K_1) and (R)-pantothenate (vitamin B_5), compared to controls. In contrast, several metabolites demonstrate diminished secretion levels into the blood in remission CeD relative to both controls and active CeD patients. These metabolites include certain amino acids (such as L-methionine and L-asparagine), vitamins (such as

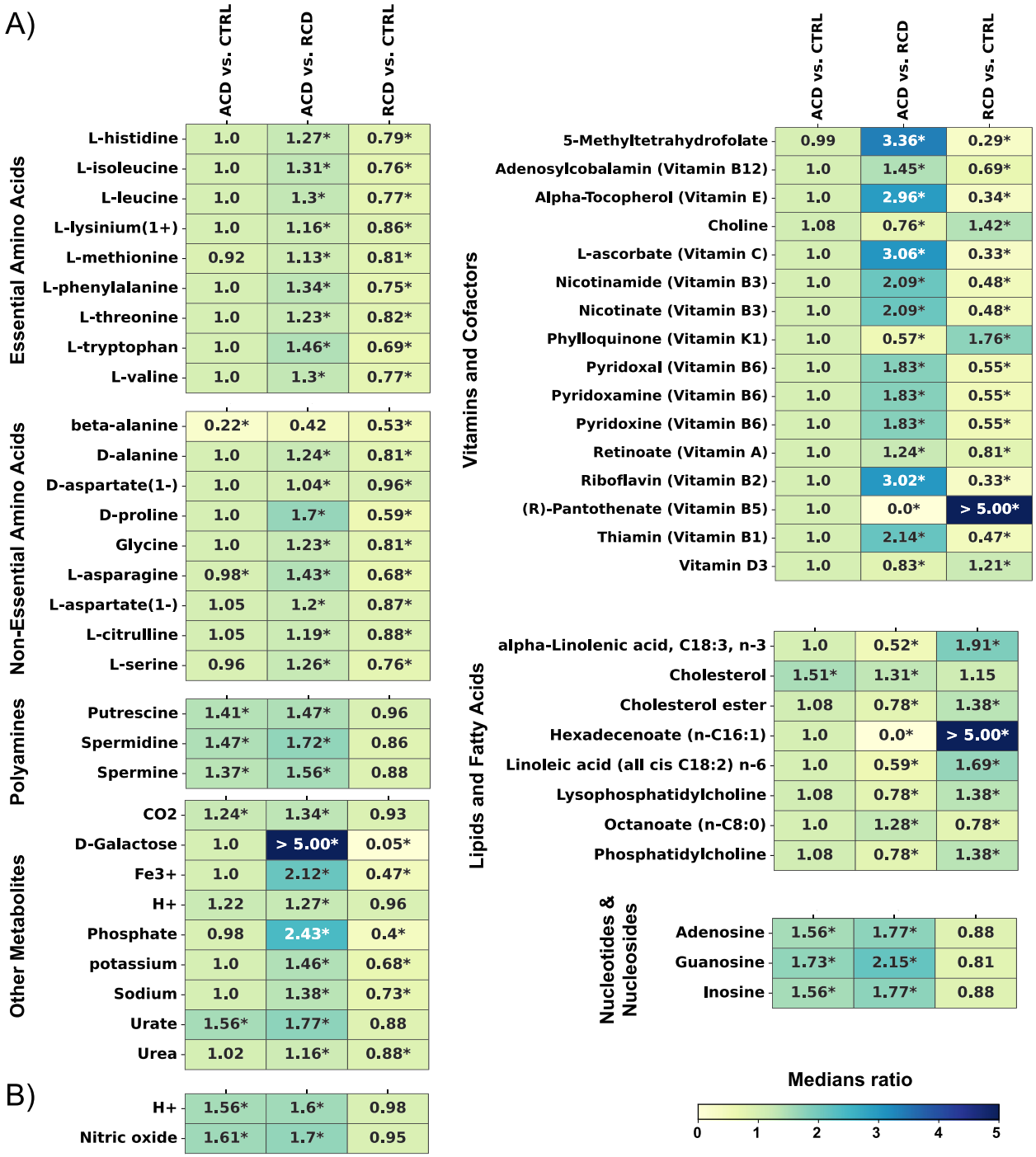

Fig. 3 Metabolites showing differential secretion profiles into the bloodstream and intestinal lumen across study groups. Metabolites exhibiting significant differential secretion into the **A** bloodstream and **B** intestinal lumen between at least one pair of study groups are shown. Heatmap annotations denote the ratio of medians in the two study groups (ACD/CTRL, ACD/RCD, and RCD/CTRL), and asterisks indicate statistical significance (Mann–Whitney *U*, $q < 0.05$). The full list of metabolites and the corresponding *q*-values are provided in Additional File 1. ACD, active CeD; RCD, remission CeD; CTRL, controls

5-methyltetrahydrofolate and adenosylcobalamin), polyamines (spermine, spermidine, and putrescine), and nucleosides (adenosine, guanosine, inosine).

Probing sIECs metabolic adaptability for performing essential functions

We evaluated the metabolic adaptability of sIECs to

perform the essential metabolic tasks and secrete metabolites in response to perturbations using shadow price analysis. This analysis identified significant differences in adaptability for two metabolic tasks: ornithine secretion into the blood and carboxylic acid dissociation (Mann–Whitney U, $q < 0.05$; see Additional File 2 for details).

Independent validation

To independently validate our findings, we created a multi-cohort validation dataset by combining RNA sequencing data from three external studies: Abadie et. al [9], Bragde et. al [10], and Dotsenko et. al [11]. These studies provided whole-genome transcriptomic data from duodenal biopsies of subjects with active CeD, CeD in remission, and non-CeD controls. Given that these studies recruited subjects from different geographic locations—Abadie et al. in the United States and Bragde et al. and Dotsenko et al. in Europe—the ComBat-seq pipeline [40] was applied to minimize batch effects prior to combining datasets.

Using this multi-cohort validation dataset, personalized GEMs were constructed for a total of 71 active CeD patients, eight remission CeD patients, and 66 control subjects. To reflect dietary differences between cohorts, an Average American and Average European diet for active CeD and control subjects from the US and Europe were used for simulations, respectively. Additionally, a European gluten-free diet was sued for subjects with remission CeD from the Dotsenko et al. study [11]. To assess the reproducibility of our results, the concordance in the direction of change (increase or decrease) between essential sIECs metabolic tasks showing significant alterations in our primary analysis (Fig. 2) and the same tasks in the validation dataset was evaluated. A concordance rate of 70.59% was observed for metabolic tasks showing significant differential activity between active CeD and controls, 25.93% for active CeD vs. remission CeD, and 66.67% for remission CeD vs. controls. A similar analysis for metabolites exhibiting significant secretion into the blood resulted in concordance rates of 81.82% for active CeD vs. controls, 40.35% for active CeD vs. remission CeD, and 43.75% for remission CeD vs. controls.

Of note, an independent analysis was also performed to identify the essential metabolic tasks showing significant differential activity between the study groups in the validation dataset alone. The results of this analysis are provided in the Additional File 2.

Drug target analysis

Potential drugs that can restore the altered activity of the differentially active metabolic tasks in CeD patients to those of healthy ones were next explored. Out of the 28 differentially active metabolic tasks, we focused our attention on six differentially active metabolic tasks which contribute to tissue destruction and CeD development. These tasks include citrate synthase, aspartate transaminase, and malate dehydrogenase (promoting pro-inflammatory immune cell activation), as well as nitric oxide synthase, xanthine oxidase, and argininosuccinate lyase (implicated in ROS synthesis) (see the previous sections and the "Discussion" section for details). Gene-protein-reaction rules in GEMs were assessed to determine the gene or genes that are responsible for each of these metabolic tasks. This resulted in seven genes (MDH, NOS1, NOS2, NOS3, CS, ASL, XDH) to serve as candidate drug targets.

Flux coupling analysis (FCA) [27] was additionally conducted to pinpoint reactions in the GEMs that are fully coupled with the reactions representing these six metabolic tasks. Modulating the expression of the genes encoding reactions that are fully coupled with reactions for a metabolic task will impact the activity of that metabolic task the same way. This analysis revealed two reactions that are fully coupled with two of the six metabolic tasks: argininosuccinate synthase, encoded by gene *ASS1*, which is fully coupled with argininosuccinate lyase, and aspartate-glutamate mitochondrial shuttle, encoded by two genes, *SLC25A12* and *SLC25A13*, and fully coupled with aspartate transaminase. This yielded a total of ten genes to target.

The DrugBank database was then queried to identify potential drugs–including both FDA-approved and those in clinical trials—that target these 10 genes by down- or up-regulating their expression levels. With this analysis, we identified FDA-approved 22 drugs that target the expression of at least one gene responsible for the six metabolic tasks or reactions fully coupled to them (Fig. 4, see Additional File 1 for the full list of all drugs). Of note, five of these drugs (acetaminophen, cisplatin, cyclosporine, silicon dioxide, valproic acid) target multiple genes.

Discussion

In this study, we sought to systematically dissect the metabolic landscape of sIECs in CeD by leveraging genome-scale modeling. To this end, patient-specific GEMs of sIECs metabolism were constructed by utilizing a manually curated base sIECs GEM and incorporating transcriptional profiles from 42 subjects with active CeD, CeD in remission, and healthy controls as well as sex-specific parameters into our analysis. By simulating a relevant diet for each group of subjects (an average American diet for active CeD patients and controls and a gluten-free diet for remission CeD subjects) using these personalized GEMs, we computationally investigated the capability of sIECs to perform 59 essential metabolic

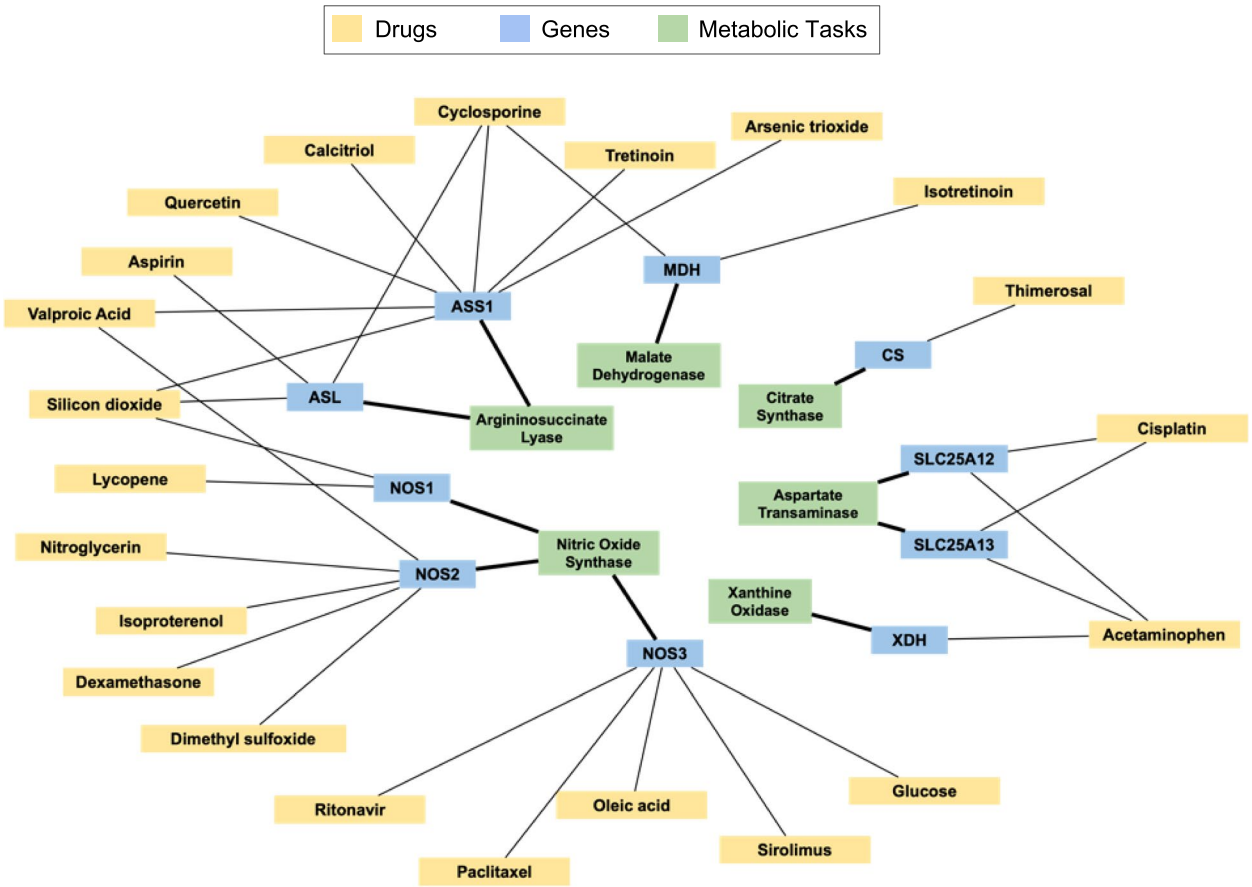

Fig. 4 Drugs targeting genes linked to altered sIECs metabolic tasks in CeD. Existing FDA-approved drugs that target 10 genes encoding six differentially active metabolic tasks in sIECs, which contribute to CeD development or symptoms, or reactions fully coupled to them, are shown. Lines represent gene-to-reaction links or gene-drug interactions. All drugs shown suppress the expression of the genes linked to them. The full list of all identified drugs (including those that are not FDA-approved) can be found in Additional File 1

functions and secrete metabolites into the bloodstream and intestinal lumen.

Active CeD patients exhibit a unique metabolic profile for essential metabolic tasks

Analyzing the overall metabolic landscape of the study individuals with regard to the essential metabolic tasks using PCA revealed a distinct metabolic state in active CeD patients compared to those with CeD in remission and healthy controls (Fig. 1C). Additionally, we observed a marked overlap between the clusters for healthy and remission CeD individuals indicating the intestinal healing that occurs within CeD patients after adopting a gluten-free diet. While the remission CeD and active CeD groups show a pronounced stratification, there is a slight interspersion between the two (near the center of PC1), which could indicate the potential of a few remission CeD patients to experience relapse and re-develop CeD symptoms despite adhering to a gluten-free diet.

Differential activity in essential metabolic tasks highlights key processes in CeD pathogenesis

We next investigated the activity of each essential metabolic task across the three study groups. This analysis revealed 28 unique metabolic tasks showing significantly altered activity between at least one pair of the study groups (Fig. 2). Many of these differentially active metabolic functions are implicated in key processes involved in CeD pathogenesis such as gut barrier maintenance, immune system modulation, and nutrient absorption and metabolism. A subset of these tasks which exhibited elevated activity in active CeD patients contribute to CeD pathogenesis by inducing a pro-inflammatory response (aspartate transaminase, malate dehydrogenase, and citrate synthase) or by compromising the gut barrier integrity via ROS production (nitric oxide synthase, xanthine oxidase, and argininosuccinate lyase).

Elevated activity in protective metabolic tasks may reflect a compensatory response in active CeD

We also identified several other metabolic tasks with elevated activity in active CeD patients that were known from existing literature to have protective effects. Notably, some of these tasks can promote anti-inflammatory responses and/or improve gut barrier integrity (malic enzyme, superoxide dismutase, transketolase 2, desmosterol reductase, spermine synthase, spermidine synthase, GMP synthase, and 2-keto-4-methylthiobutyrate transamination). This seemingly counterintuitive observation for these metabolic tasks highlights the complex relationships between these critical metabolic functions and the pathophysiology of CeD. A highly likely scenario for the heightened activity of the metabolic tasks with protective effects in the active CeD patients may represent a compensatory response by the body to mitigate intestinal damage due to the immune response to gluten. Other factors that may also contribute to these observations may include the malabsorption of nutrients due to intestinal damage, the disruption of metabolic pathways triggered by disease, or the complex interactions between multiple metabolic processes in the disease state. These insights underscore the multifaceted nature of metabolic adaptations in response to the physiological challenges imposed by CeD.

Altered mitochondrial functions in active CeD indicate shifts in energy metabolism and inflammatory response

The elevated activity of mitochondrial aspartate transaminase, malic enzyme, malate dehydrogenase, citrate synthase, and riboflavin kinase observed in active CeD points to notable alterations in mitochondrial energy metabolism. Elevated blood levels of aspartate transaminase, which is commonly used as a marker for liver health [40], has been reported in untreated CeD patients [41, 42], suggesting that it may be associated with inflammation or pro-inflammatory response in CeD. This aligns with prior evidence that aspartate metabolism promotes IL-1β production in M1 macrophages [43]. Transaminase activity was reported to return to normal levels after following a gluten-free diet [42, 44]. Interestingly, the co-occurrence of CeD with autoimmune liver diseases has been documented before, strengthening the possibility of shared inflammatory pathways between the two [45].

Increased flux through reactions for malic enzyme, malate dehydrogenase, and citrate synthase reflects the heightened energy demands in active CeD, as these enzymes play key roles in ATP production through the TCA cycle. Malate dehydrogenase and citrate synthase are also linked to pro-inflammatory responses and macrophage and dendritic cell activation [46, 47]. Conversely, malic enzyme supports protective functions such as promoting sIECs proliferation and barrier maintenance [48]. Riboflavin kinase also plays a vital role in producing FMN and FAD, essential cofactors in the TCA cycle and electron transport chain [49]. The increased activities of malic enzyme and riboflavin kinase potentially serve as a compensatory response to the energy deficit caused by nutrient malabsorption and intestinal damage.

Elevated ROS-related metabolic activities in active CeD reflect oxidative stress and antioxidant defense responses

Three metabolic tasks contributing to ROS production (nitric oxide synthase, xanthine oxidase, argininosuccinate lyase) and three others engaging in ROS metabolism (superoxide dismutase, transketolase 2, and desmosterol reductase) showed significantly increased activity in active CeD compared to remission CeD and controls (Fig. 2). ROS are highly reactive molecules that can cause oxidative damage and apoptosis in the intestine, compromising gut barrier integrity. They also impact the immune response, for example, by activating macrophages and other innate immune cells towards pro-inflammatory states and have been implicated in many diseases involving chronic inflammation [50]. High levels of ROS are a biomarker of CeD and play a role in its pathogenesis by contributing to villi damage [51].

Nitric oxide synthase activity as well as nitric oxide levels are reported to increase in CeD patients in response to gluten but decrease upon adopting a gluten-free diet [52, 53]. Xanthine oxidase has been shown to polarize immune cells into a pro-inflammatory phenotype [54]. Elevated activity of argininosuccinate lyase, also linked to nitric oxide production, exacerbates oxidative damage, and inflammation in enterocytes [55], although it is also associated with improved epithelial integrity and decreased apoptosis in other contexts, such as in vitro models of colitis [56] and necrotizing enterocolitis [57].

The observed increased activity of the three ROS-metabolizing enzymes (superoxide dismutase, transketolase 2, and desmosterol reductase) in active CeD, which mitigate oxidative stress (see Additional File 2 for details), aligns with prior studies. For example, superoxide dismutase elevated activity has been documented in active CeD patients [58]. It was proposed that gliadin may cause an imbalance in antioxidant activity, leading to the increased likelihood of lesions in the intestinal mucosa [59]. The increased activity of these enzymes may also represent the body's response to counterbalance the enhanced activity of nitric oxide synthase and xanthine oxidase in active CeD patients.

Heightened polyamine synthesis in active CeD may represent an intrinsic protective response to counteract inflammation and maintain gut integrity

Spermine synthase and spermidine synthase, which are involved in synthesizing polyamines, show elevated activity in active CeD compared to remission CeD and healthy controls (Fig. 2). Spermine, the product of spermine synthase, and its precursor spermidine play important roles in supporting epithelial growth and maintaining gut barrier integrity [34] as well as modulating immune responses. Spermine has also been characterized as an inhibitor of inflammation through protecting against oxidative stress and inhibiting inflammatory cytokine synthesis within the innate immune system [60]. Similarly, spermidine has been shown to attenuate gliadin's toxic effects on sIECs [61], induce $CD4^+$ T cell differentiation towards regulatory T cells (Treg) suppressing pro-inflammatory responses, and polarize macrophages towards an anti-inflammatory phenotype [60, 62].

The elevated activity of these protective metabolic functions observed in our study is consistent with existing literature for other inflammatory diseases. For example, spermine, along with other polyamines, were reported to be elevated in the blood serum and colonic mucosa of colorectal cancer patients [63] and in patients with acute colitis [62, 64]. Interestingly, spermidine is also currently being explored as a therapy to inhibit tTG (tissue transglutaminase) activity, inhibiting inflammation due to gliadin deamidation [65]. These findings suggest that elevated spermine and spermidine synthesis may represent an adaptive response by the body to mitigate the inflammation and damage to the intestine in active CeD.

Altered activity in nucleotide synthesis and DNA repair functions reflects heightened cellular repair demands and disrupted metabolic pathways in active CeD due to tissue damage

Five metabolic tasks related to nucleotide synthesis and DNA repair showed significant differences in activity across the study groups. In active CeD, the observed heightened activity in adenylosuccinate lyase, adenine phosphoribosyltransferase, and fumarase is likely due to increased DNA repair and synthesis demands resulting from extensive cellular damage in the intestine. Conversely, the reduced activity of B-ureidopropionase in active CeD might indicate disrupted pyrimidine metabolism, suggesting significant metabolic reprogramming in response to cellular stress and damage during active disease. Additionally, the decreased activity of thymidylate synthase in remission CeD could reflect a reduced need for DNA synthesis and repair as intestinal healing progresses under a gluten-free diet.

Altered amino acid metabolism in active and remission CeD reveal disease-specific metabolic adjustments

Our analysis identified significant changes in amino acid metabolism and transport across the study groups, including elevated secretion of ornithine, L-proline, and L-arginine in active CeD. This aligns with previous reports of increased plasma amino acid levels in active CeD patients [39, 66]. CeD patients have been shown to have impaired capacity for absorbing amino acids and peptides compared to healthy individuals due to the damage to intestine [67], potentially contributing to increased activity of enzymes involved in amino acids synthesis. Furthermore, it has been suggested that the elevated plasma levels of amino acids in active CeD patients may contribute to the systemic inflammation seen in CeD [39].

Among amino acid-related metabolic tasks, L-alanine transaminase, 5, 10-methylenetetrahydrofolate reductase (NADPH), and arginase showed significantly reduced activity in remission CeD compared to active CeD and controls. While prior research has not investigated the activity of these functions in remission CeD, a number of studies have reported their elevated activity in active CeD [41, 68], a trend we do not observe in our results. The reduced activity of these enzymes in remission CeD may be attributed to the nutritional constraints of the gluten-free diet, which can alter amino acid metabolism.

GMP synthase and 2-keto-4-methylthiobutyrate transamination are two other metabolic tasks involved in L-glutamate production that demonstrated increased activity in active CeD. Elevated plasma glutamate levels have been documented in active CeD [39] and are associated with improved intestinal barrier function and antioxidant defense, helping reduce injury to the intestine caused by inflammation [69].

Genome-scale modeling reveals insights into sIECs metabolism beyond gene expression changes

Notably, for numerous metabolic functions that displayed significant differential activity, we did not observe any significant changes in the expression levels of the genes encoding them. This highlights the merits of employing genome-scale modeling in elucidating the phenotypic outcomes that arise from the complex interplay among multiple metabolic pathways. This system-wide perspective offers non-intuitive insights that transcend those provided by the isolated examination of expression levels for individual genes. GEMs thus deliver a more holistic understanding of sIECs metabolism and function.

Differential metabolite secretion profiles highlight metabolic alterations in sIECs across the study groups

In addition to the differentially active essential metabolic tasks, we identified 58 distinct metabolites with significant differential secretion into blood and two into the lumen between at least one pair of the study groups. Notably, we observed elevated secretion of polyamines—spermine, spermidine, and putrescine—and urate into the blood and nitric oxide into the lumen in active CeD compared to controls and remission CeD patients. Although the increased secretion of spermine, spermidine, and putrescine in active CeD observed in our study has not been previously reported, elevated blood levels of these polyamines have been noted in other autoimmune diseases [70, 71]. Furthermore, the increased secretion of spermine and spermidine in active CeD is consistent with the heightened activity of the essential metabolic tasks spermine synthase and spermidine synthase in our study (Fig. 2). Likewise, the increased secretion of the ROS nitric oxide in active CeD is consistent with the elevated activity of nitric oxide synthase in active disease (Fig. 2). In contrast to nitric oxide, urate is an antioxidant and is reported to have higher serum concentration in adults with CeD, which aligns with our findings [72]. We additionally observed the reduced secretion into the blood of certain vitamins and essential nutrients including (R)-pantothenate (vitamin B_5), vitamin D_3, and choline in active CeD relative to remission CeD, which is consistent with micronutrient deficiencies often observed in CeD patients [73] due intestinal damage. Finally, the significantly altered secretion profiles for several essential and non-essential amino acids into the blood in both active CeD and remission CeD were observed, which aligns with significant alterations in several essential metabolic tasks implicated in amino acids synthesis and metabolism (Fig. 2). Overall, these observed variations in metabolite secretion profiles reflect alterations in sIECs metabolism due to the disease state and/or dietary interventions.

Altered metabolic activity and secretion profiles in remission CeD reveal nutritional and physiological adaptations

A number of metabolic tasks, including L-alanine, L-arginine, and L-proline secretion as well as L-alanine transaminase, 5 10 methylenetetrahydrofolate reductase NADPH, arginase, and thymidylate synthase showed significantly diminished activity or secretion in remission CeD compared to both controls and active CeD subjects. The same pattern was also observed for the secretion of several metabolite into the bloodstream such as amino acids L-ascorbate, L-methionine, and L-citrulline), vitamins 5-methyltetrahydrofolate (vitamin B_9) and adenosylcobalamin (vitamin B_{12}), polyamines spermine, spermidine, and putrescine, and nucleosides guanosine and inosine. Some of these metabolic tasks and secreted metabolites play key roles in modulating inflammation (guanosine and inosine [74, 75]), immune response (spermine and spermidine), oxidative stress (vitamin B9: 5-methyltetrahydrofolate and vitamin C: L-ascorbate), or DNA repair (thymidylate synthase). Their reduced activity or secretions suggests a reduction in inflammatory and immune responses and the need for intestinal repair as patients transition into remission. This trend can be also attributed, in part, to the nutritional deficiencies inherent to the gluten-free diet, which could alter metabolic pathways and secretion profiles in these subjects. This phenomenon could additionally stem from a combination of other inter-connected factors. These may include incomplete intestinal healing in remission CeD (where enterocyte function remains suboptimal), persistent residual inflammation influencing metabolic pathways, or the complex adaptive regulatory mechanisms in remission CeD aimed at managing persistent residual inflammation through adjusting metabolic and transporter activities. These elements reflect the complex interplay of nutritional factors, physiological adaptations, and residual effects even in the remission phase of CeD.

Targeting dysregulated sIECS metabolic functions in CeD through FDA-approved drugs provides potential therapies for CeD beyond dietary restrictions

Following a gluten-free diet is currently the only available treatment for CeD, which is very difficult to undertake as gluten is present in the vast majority of foods. In this study, we conducted a drug target analysis, which identified 22 FDA-approved drugs that target genes encoding six differentially active metabolic tasks that adversely affect sIECs function and contribute to intestinal damage and CeD pathogenesis. By restoring the normal expression levels of these target genes and consequently rectifying the activity of the respective metabolic tasks, these medications offer promising therapeutic options for ameliorating CeD symptoms. This can especially benefit patients that persistently suffer despite adhering to a gluten-free diet [76] or those who cannot fully maintain such a strict diet. Several of these drugs have immunosuppressive or anti-inflammatory properties. In particular, cyclosporine, dexamethasone, sirolimus, and isotretinoin have been approved by the FDA as anti-inflammatory or immunosuppressant drugs in multiple disease contexts such as rheumatoid arthritis and cancer [25]. Additionally, two of these drugs, cyclosporine and methotrexate, have been investigated to treat other inflammatory diseases of the gut such as Crohn's disease and ulcerative colitis [77, 78]. However, none of these medications are currently being investigated to treat symptoms of

CeD. Other drug groups identified in this study include chemotherapy drugs and other anti-tumor agents, corticosteroids, retinoids as well as common over-the-counter vitamins and supplements. Of these, corticosteroids such as prednisolone have been investigated to potentially aid CeD patients who do not improve upon starting a gluten-free diet [79]; however, the corticosteroid dexamethasone, which was identified in this analysis, has not yet been studied in the context of CeD.

Independent validation using external datasets highlights the influence of dietary and regional factors in shaping sIECs metabolic responses in CeD

To further strengthen the reliability of our findings beyond validation based on existing literature evidence, we performed an independent validation using combined external datasets from multiple cohorts. This analysis provided additional insight into the robustness of our results and revealed both consistencies and variations. Particularly, the high directional concordance rate between our findings and the validation dataset for active CeD vs. controls comparisons highlights the consistency of key metabolic disruptions in active CeD across diverse patient cohorts. On the other hand, the lower concordance rates for the two comparisons involving remission CeD are likely due to the very limited sample size of remission CeD subjects in the validation dataset, dietary differences between the gluten-free diets used in the US (for Leonard et al. [7]) and Europe (for Dotsenko et al. [11]), and/or residual batch effects between the Leonard et al. dataset and the combined validation dataset that we observed even after ComBat-seq correction (Additional File 3: Fig. S2). This suggests that our results for comparisons involving remission CeD may be influenced by cohort-specific factors and require further validation in additional external datasets. This independent validation enhances the overall reliability of our findings while also highlighting key areas for further investigation and improvement.

Limitations of this study

While GEMs of metabolism are powerful tools for modeling metabolic reactions and the interactions between reaction products and metabolic pathways, it is essential to acknowledge their inherent constraints and limitations. Notably, GEMs do not encompass regulatory interactions, allosteric regulation of enzymes, and the effects of cell signaling and cytokine production, among others, all of which can influence the complex metabolic landscape of the human intestine. Additionally, human metabolism is a complex system involving intricate interactions between various organs and tissues. While this study focused specifically on sIECs metabolism, a more comprehensive analysis—such as using organ-resolved whole-body models of metabolism [23]—would be necessary to capture the effects of inter-organ and microbiome-host interactions on sIEC metabolism in CeD. Furthermore, while our findings provide valuable insights, their generalizability to other patient cohorts may be influenced by factors such as dietary differences, cohort composition, and regional variability. The predictions derived from the personalized sIECs GEMs thus require subsequent validation through literature reviews, in vitro, or in vivo studies, and ultimately clinical trials.

While these limitations highlight the need for complementary experimental and clinical studies, GEMs nonetheless provide a robust framework for hypothesis generation and prioritization of promising dysregulated metabolic functions for further investigation or therapeutic targeting. For example, our findings for disrupted essential metabolic functions or candidate FDA-approved drugs provide a solid basis for targeted experiments using patient-derived gut organoid or gut chip models. Recent research employing patient-derived gut organoid models to explore the protective role of a strain of *Bacteroides vulgatus* in ameliorating intestinal damage in CeD [80] exemplifies the potential of these experimental systems to validate GEM predictions.

Conclusions

This study presents a rigorous and quantitative exploration of sIECs metabolic dysfunction in CeD by leveraging highly personalized GEMs of metabolism that incorporate patient-specific transcriptional data and sex-specific parameters. As demonstrated, this approach goes beyond classical differential gene expression analysis, enabling the observation of intricate interactions within the complex network of metabolites, enzymes, metabolic reactions, and pathways in disease. Our findings provide new insights into the dysregulation of specific metabolic processes within sIECs in CeD, shedding light on novel avenues for therapeutic intervention and personalized treatment strategies. Specifically, this research has pinpointed promising FDA-approved drug candidates potentially capable of rectifying the disrupted metabolic functions contributing to CeD development and CeD-associated tissue destruction, offering a pathway to repurposing existing drugs for CeD treatment. While these insights provide a strong foundation for future research, the predictions derived from GEMs require further validation through experimental and clinical studies. In particular, biological validation using systems such as patient-derived gut organoids, gut chips, or animal models will be critical to confirm the therapeutic potential of the identified metabolic targets and drug candidates. Notably, the

integrative approach utilized in this study has broad applicability and could be extended to other chronic and inflammatory conditions, enabling a deeper understanding of metabolic dysfunction in various disease contexts.

Abbreviations

CeD	Celiac disease
sIECs	Small intestinal epithelial cells
GEM	Genome-scale model
COBRA	Constraint-Based Reconstruction and Analysis
FBA	Flux Balance Analysis
WBM	Whole-body model

Supplementary Information

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Additional file 1. Detailed diet information, predicted fluxes for the essential metabolic tasks and metabolite secretions, and results from various analyses including PCA with standardized data, shadow price analysis, statistical analysis, and drug target analysis.

Additional file 2. Details on certain essential metabolic tasks, shadow price analysis, sensitivity analysis, validation of findings using independent external datasets, and analysis of essential metabolic tasks within the validation dataset.

Additional file 3: Figs. S1 and S2. Fig. S1—Differential expression analysis of genes related to differentially active essential metabolic tasks. Fig. S2—PCA on gene expression data before and after batch correction.

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Authors' contributions
ARZ conceived the study and, together with CVM, interpreted the results and drafted the manuscript. CVM performed all analyses. DA conducted the whole-body model simulations. KM designed the list of food items for the average gluten-free diet. AF provided critical feedback on the results and manuscript. All authors have read and approved the final manuscript.

Authors' Twitter handles
Twitter handles: ARZ: @arzomorrodi.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
N/A.

Consent for publication
N/A.

Competing interests
A patent application arising from this work by ARZ, CVM, and AF is currently pending review. The rest of the authors declare no competing interests.

Author details
[1]Department of Biological Engineering, MIT, Cambridge, MA, USA. [2]Mucosal Immunology and Biology Research Center, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA. [3]Harvard Medical School, Boston, MA, USA. [4]John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA. [5]Boston University School of Medicine, Boston, MA, USA. [6]The Center for Celiac Research and Treatment, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA.

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