MYC mRNA expression throughout the intestine is not associated with body mass index or type 2 diabetes

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1 | BACKGROUND

In most parts of the world, the prevalence of obesity and other metabolic disorders such as type 2 diabetes is increasing. These conditions typically carry large personal and social consequences. The underlying pathophysiological processes remain obscure, and unfortunately, available treatment options are sparse. Thus, the exploration of new therapeutic targets has become a major focus area for researchers and the pharmaceutical industry driving the development of new and effective drugs for the treatment of metabolic disorders.

Recently, Luo et al. proposed intestinal MYC as a putative drug-gable target against metabolic diseases. MYC is a transcription factor with broad biological function involved in cellular homeostasis and proliferation and is characterised as a proto-oncoprotein. Among its multiple actions are the regulation of energy metabolism, and the MYC transcription factor network has been implicated in diabetes and other metabolic diseases. Luo et al. showed that individuals with overweight (body mass index [BMI] > 25 kg/m²) had significantly elevated MYC mRNA expression in distal ileum mucosa biopsies compared to individuals with normal weight (BMI < 25 kg/m²). Furthermore, BMI and distal ileum MYC mRNA expression correlated positively. The results were reproduced in mice fed a high-fat diet vs. a chow diet; and with a plethora of data, the study convincingly shows that improved metabolic health can be obtained in high-fat-fed mice by decreasing MYC in the intestine (genetically or with the MYC inhibitor 10058-F4). In a gene expression network analysis, Vargas et al. showed a link between BMI and intestinal MYC in rectal mucosa biopsies from premenopausal women. This study shows that intentional weight loss results in a regulatory shift towards transcription of more MYC-regulated targets; thus, indicating that MYC-dependent transcription correlates positively with BMI. However, whether the expression level of MYC mRNA itself changes in the rectal mucosa upon intentional weight loss was not reported. Collectively, intestinal MYC in these very distinct parts of the intestinal tract (distal ileum and rectum) presents itself as a new putative drug target against obesity and other metabolic diseases.

Here, we investigated mucosal MYC mRNA expression along the entire intestinal tract in patients with type 2 diabetes and matched healthy controls to elucidate (1) the MYC mRNA expression profile along the intestinal tract of humans and (2) whether intestinal MYC mRNA expression correlates to BMI and/or glycaemic control (HbA1c level).

2 | METHODS

2.1 | Biopsy retrieval and transcriptomics

The study design, the study population, the experimental procedures for biopsy retrieval and storage, as well as the transcriptomic analysis have been previously described. In brief, 12 patients with type 2 diabetes (treated using diet-counselling alone or in combination with metformin or sulfonylurea) and 12 healthy controls (without first-degree relative with type 1 or type 2 diabetes) matched on age, gender, and BMI were included in the study after obtaining of informed consent. Demographical details are presented in Table 1. During propofol sedation, subjects underwent anterograde and retrograde double-balloon enteroscopies (on two separate days) including the collection of biopsies from every 30 cm throughout the small intestine and from the cecum, the ascending, transverse, descending, and sigmoid large intestine, as well as the rectum. The biopsies were subjected to mRNA extraction, cDNA synthesis, and cDNA sequencing using the NextSeq 500 system (Illumina, San Diego, CA, USA).

2.2 | Statistical analyses

To best compare our data with the previous results and to account for possible variation between biopsy sample sites between subjects, we pooled MYC mRNA expression data from several biopsy sample sites into four main categories: proximal small intestine, distal small intestine, proximal large intestine, and distal large intestine. Thus, for each subject, a mean MYC mRNA expression was calculated for each of the four intestinal sections and used for the analyses. To evaluate any differences in MYC expression level, the mean value for each section in the two groups (control and type 2 diabetes) were compared using two-way ANOVA followed by Šidák’s multiple comparisons test. Correlations were evaluated with nonparametric Spearman’s correlation test. p-values were calculated with

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levels in the proximal small intestine and in the colon (Figure 1A). Observed at the distal small intestine and the greatest expression in the large intestine (Figure 1B, D, E). Similarly, we observed no difference in expression in the proximal small intestine or in the proximal or distal ileum mucosa biopsies reported by Luo et al.1 (biopsies sampled at a hospital in Shanghai and, thus, presumably a population predominantly of Asian origin) and by Vargas et al.5 (study conducted in the US on subjects with different racial backgrounds) may explain the discrepant findings. Furthermore, different methodology to determine MYC mRNA expression was used in the different studies, thus, complicating direct comparisons. Lastly, the MYC mRNA level does not necessarily reflect the level of the functional protein, and thus, we cannot exclude a difference in functional MYC between patients with type 2 diabetes and healthy controls. To address this, a full stereological analysis is needed.

In conclusion, we do not see a correlation between intestinal mucosal MYC mRNA expression and BMI or glycaemic control, and thus, our findings do not support the hypothesis of intestinal MYC as a putative drug target against obesity and metabolic diseases. However, we cannot rule out that the discrepancies between the results presented here and in the literature1,5 arise due to lack of statistical power in our dataset and/or due to racial and/or methodological differences.

**KEYWORDS**
mRNA expression, obesity, type 2 diabetes

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### TABLE 1 Demographics of participants with type 2 diabetes and healthy individuals.

| Variable                  | Type 2 diabetes | Healthy control | p-value |
|---------------------------|-----------------|-----------------|---------|
| Sex (M/F)                 | 9/3             | 8/4             | –       |
| Age (years)               | 51 (34–63)      | 50 (41–67)      | 0.66    |
| BMI (kg/m²)               | 26.8 (23.7–31.5) | 27.1 (20.3–30.8) | 0.92    |
| HbA1c (%)                 | 6.5 (5.4–9.9)   | 5.3 (4.8–6.1)   | –       |
| HbA1c (mmol/mol)          | 48 (36–85)      | 34 (29–43)      | –       |
| Duration of type 2 diabetes (years) | 5.0 (1.0–9.0) | –              | –       |
| Diabetes treatment        |                 |                 |         |
| Metformin alone           | 9               | –               | –       |
| Sulfonylurea alone        | 2               | –               | –       |
| Metformin + Sulfonylurea  | 1               | –               | –       |

Note: Data are presented as means (ranges). p-values reflect the results of Student’s t-tests used to evaluate the match between groups. Table modified from Jorsal et al.8

Abbreviation: BMI, body mass index.

95% confidence intervals and considered statistically significant at \( p < 0.05 \). GraphPad Prism 9.0.0 (GraphPad software, San Diego, CA, USA) was used for the statistical analyses.

### 3 | RESULTS

Inspired by the prospect of intestinal MYC reduction as a putative drug target against metabolic disorders, we investigated MYC mRNA expression in intestinal mucosa biopsies sampled using double-balloon enteroscopy along the entire intestinal tract of 12 patients with type 2 diabetes and 12 matched healthy controls.6,7 In these individuals with BMI ranging from 20 to 31 kg/m², the mean MYC mRNA expression varied along the intestine with the lowest level observed at the distal small intestine and the greatest expression levels in the proximal small intestine and in the colon (Figure 1A).

Intestinal MYC is described as a driver of metabolic diseases.1 Hence, we investigated whether MYC mRNA expression levels were increased in intestinal biopsies from patients with type 2 diabetes. We observed no differences between the groups at any of the examined sites (Figure 1A), and thus, increased intestinal MYC mRNA expression does not seem to be implicated in the pathophysiology of type 2 diabetes. The positive correlation between BMI and MYC mRNA expression in distal ileum mucosa biopsies reported by Luo et al.2 could not be replicated in our dataset (Figure 1C). Neither did we observe significant correlations between BMI and MYC mRNA expression in the proximal small intestine or in the proximal or distal large intestine (Figure 1B, D, E). Similarly, we observed no correlation between glycaemic control (HbA1c) and MYC mRNA expression at any of the four sites (Figure 1F–I).

### 4 | CONCLUSIONS

We show a differentiated expression profile of MYC along the intestinal tract in humans; with lowest MYC mRNA expression in the distal small intestine. This indicates a variation in cellular dependency on MYC-regulated transcription throughout the gut, and thus, implies that the findings by Luo et al.2 and Vargas et al.5 may reflect blinkered insights into the role of intestinal MYC expression in metabolic disorders. Notably, the genetically and inhibitor–induced reductions in MYC levels in mice scrutinised by Luo et al.1 supposedly affected MYC throughout the entire intestine and are as such independent of the MYC expression site. Furthermore, we show no difference in MYC mRNA expression between patients with type 2 diabetes and healthy controls, and lastly, we cannot reproduce the association between MYC mRNA levels and BMI as observed by Luo et al.1 and indicated by Vargas et al.5

Importantly, our data set was not obtained for these analyses, and thus, the number of subjects might be too low to detect significant correlations; however, no trends seem apparent in any of the plots (Figure 1B–E). Also, differences between the participants included in our study (Danish subjects with Northern European origin) and the participants examined by Luo et al.1 (biopsies sampled at a hospital in Shanghai and, thus, presumably a population predominantly of Asian origin) and by Vargas et al.5 (study conducted in the US on subjects with different racial backgrounds) may explain the discrepant findings. Furthermore, different methodology to determine MYC mRNA expression was used in the different studies, thus, complicating direct comparisons. Lastly, the MYC mRNA level does not necessarily reflect the level of the functional protein, and thus, we cannot exclude a difference in functional MYC between patients with type 2 diabetes and healthy controls. To address this, a full stereological analysis is needed.

In conclusion, we do not see a correlation between intestinal mucosal MYC mRNA expression and BMI or glycaemic control, and thus, our findings do not support the hypothesis of intestinal MYC as a putative drug target against obesity and metabolic diseases. However, we cannot rule out that the discrepancies between the results presented here and in the literature1,5 arise due to lack of statistical power in our dataset and/or due to racial and/or methodological differences.
LETTER TO THE EDITOR

CONFLICT OF INTERESTS
None of the authors reports conflicts of interest in relation to the present paper.

AUTHOR CONTRIBUTION
Anne-Marie Elleegaard: Conceptualization (supporting); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (supporting); Project administration (equal); Resources (supporting); Visualization (lead); Writing – original draft (supporting); Writing – review & editing (equal).

Filip K Knop: Conceptualization (lead); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (equal); Resources (lead); Supervision (lead); Visualization (supporting); Writing – original draft (supporting); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT
The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

FIGURE 1  MYC mRNA expression varies along the intestinal tract, is not increased in patients with type 2 diabetes and does not correlate with either body mass index or HbA1c. (A) mRNA expression levels of MYC in biopsies from the indicated intestinal sites from patients with type 2 diabetes (T2D, *n* = 12, red) and age, gender and body mass index (BMI)-matched healthy controls (*n* = 12, blue). (B–E) correlation of BMI with MYC mRNA expression level in the proximal small intestine (B), distal small intestine (C), proximal large intestine (D) and distal large intestine (E). (F–I) correlation of HbA1c level with MYC mRNA expression level in the proximal small intestine (F), distal small intestine (G), proximal large intestine (H) and distal large intestine (I). Data are presented as mean ± SEM. In each plot (B–I), the *r*-value represents the Spearman correlation coefficient and the *p*-value results from a Spearman’s correlation test of the pooled MYC mRNA expression values and either BMI (B–E) or HbA1c (F–I), respectively (presented as a purple line). RPKM, reads per kilobase million; T2D, type 2 diabetes.
LETTER TO THE EDITOR

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