

## Short Communication

# Mushroom intolerance: a novel diet–gene interaction in Crohn’s disease

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Carrying a functional single nucleotide polymorphism (L503F, c. 1672 C > T) in the gene for the Na-dependent organic cation transporter (OCTN1), increases the risk of Crohn’s disease (CD) in some, but not all, populations. Case–control data on New Zealand Caucasians show no differences for CD risk between individuals carrying the L503F OCTN1 C-allele when compared with those carrying the variant T-allele. However, more of the New Zealand CD cases report intolerance to maize and mushrooms than those who report beneficial effects or no differences. The *OCTN1* gene encodes a transporter for ergothionine, a fungal metabolite at high levels in mushrooms but not widely common in other dietary items. An inability to tolerate mushrooms showed statistically significant associations with the variant OCTN1 genotype. That is, among those individuals reporting adverse effects from mushrooms, there was a higher frequency of the variant T-allele when compared with the general population, or with CD patients overall. We believe that this is a novel gene–diet association, suggesting that individuals carrying the OCTN1 variant single nucleotide polymorphism may have an enhanced risk of adverse symptoms associated with consuming mushrooms. Nutrigenomic approaches to dietary recommendations may be appropriate in this group.

### Nutrigenomics: Crohn’s disease: Organic cation transporter 1: Single nucleotide polymorphisms: Mushrooms

Crohn’s disease (CD) is a chronic inflammatory disorder, for which strong evidence of genetic susceptibility associates with specific chromosomal locations, including IBD5 on chromosome 5q31 (MIM# 606348)<sup>(1)</sup>. This region contains the Na-dependent organic cation transporter (*OCTN1*; alternatively called *SLC22A4*). A functional single nucleotide polymorphism L503F in exon 9 of *OCTN1* (c. 1672 C > T) causes an amino acid substitution in the coding region of this gene, and the variant allele was claimed to be implicated in CD causality in a European population<sup>(2)</sup>. However, this observation has not consistently replicated across different population groups, including a preliminary study in Canterbury, New Zealand<sup>(3,4)</sup>. We suggest that such apparent contradictions of disease risk factors across different population groups might result from a gene–diet interaction.

The same dietary item that improves the condition in one individual with CD may exacerbate it in another<sup>(5)</sup>.

For example, whereas most individuals show health benefits from eating a wide range of vegetables, this is not necessarily true for all individuals with CD or other forms of inflammatory bowel disease<sup>(6)</sup>.

The main physiological substrate of the *OCTN1* is L-ergothionine (ET), which is exclusively synthesised by certain soil fungi and bacteria, and sequestered by plants. Taubert *et al.*<sup>(7)</sup> demonstrated that the L503F single nucleotide polymorphism in the *OCTN1* gene leads to a gain of function polymorphism, resulting in a 3-fold higher substrate affinity and 50% higher initial transport capacity at nanomolar levels of substrate. Because ET concentrations found in human tissues are in the nanomolar to micromolar range, these authors suggested that carriers of the variant L503F allele could accumulate higher ET concentrations in *OCTN1*-expressing cells. Thus, any potential gene–diet interactions with *OCTN1* might be anticipated to involve foods

**Abbreviations:** CD, Crohn’s disease; ET, L-ergothionine; OCTN, organic cation transporter.

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with high levels of ET. The present preliminary (negative) New Zealand case-control study on *OCTN1* and CD risk<sup>(4)</sup> has been extended to include a wider population group. We stratify their responses to vegetables, and report a novel gene-diet interaction in this New Zealand CD cohort.

## Materials and methods

### Cohort

Human subjects were a subset of a population-based inflammatory bowel disease cohort from Canterbury, New Zealand<sup>(8)</sup>, volunteers from Auckland District Health Board gastroenterology clinics or healthy volunteers from either area<sup>(9)</sup>. Diagnosis of CD was clinically confirmed using Montreal classification criteria<sup>(10)</sup>. Their phenotypes are described previously<sup>(8,10)</sup>. The study was conducted under ethical protocol MEC/04/12/011, authorised through the New Zealand Multi-Region Human Ethics Committee.

### Applied Biosystems TaqMan<sup>®</sup> single nucleotide polymorphism genotyping

DNA was extracted from peripheral blood lymphocytes using standard methods. The single nucleotide polymorphism at position c. 1672 C > T (L503F) rs1050152 was genotyped using the TaqMan MGB diallelic discrimination system, and probes and oligonucleotides were obtained from Applied Biosystems (Foster City, CA, USA) using the Assay-by-Design product. Details can be provided on request.

### Dietary questionnaire

A subject's reaction to each of a list of forty-four individual vegetables was scored on a five-point ordinal scale: ++ 'definitely better' to -- 'definitely worse'. Data for which responses were 'don't know' or 'makes no difference' were not utilised in the analysis. The response of an individual to each vegetable was summarised by two numbers:

1. the percentage of individuals reporting ++ or + 'beneficial' responses;
2. the percentage of individuals reporting -- or - 'adverse' responses.

This allowed the data to be represented in two dimensions.

### Statistical analysis

The allelic trend test<sup>(11)</sup> and Fisher's exact genotypic test were used to compare case and control allele frequencies of the *OCTN1* variant using the Statistical Analysis System software package version 9.1 (SAS Institute Inc., Cary, NC, USA). An exact test was used to test for departures from Hardy-Weinberg equilibrium<sup>(12)</sup>. The dietary questionnaire data were analysed as percentages of individuals reporting a beneficial or an adverse response, as shown in Fig. 2. The percentage data are represented on a logit scale to stabilise variance of proportions. For data from the two extreme vegetables – mushroom and maize – the regression of the proportion reporting extreme responses in each genotype against the number of variant (T) alleles was performed using logistic regression.

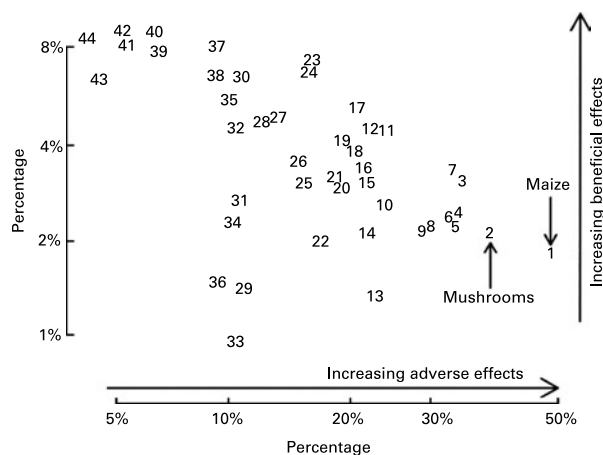
As reported, the data for mushrooms but not maize showed statistically significant evidence for an interaction ( $P < 0.025$ ).

## Results and discussion

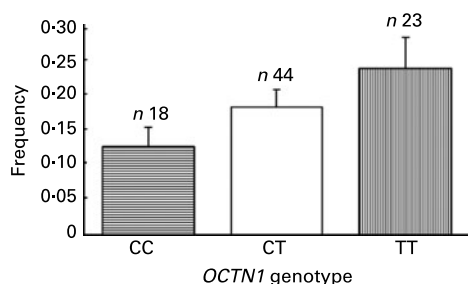
Four hundred and ninety-nine New Zealand Caucasian subjects with CD and 370 controls provided DNA samples for *OCTN1* genotyping. The case-control analysis of the present study database showed no significant differences in disease risk with increased frequency of the *OCTN1* L503F variant genotype (data not shown). A significant proportion of the New Zealand population is Caucasian, typically being descendants of migrants from the UK. The present observation confirms data from our preliminary study<sup>(4)</sup>, suggesting that variants in this gene are not associated with CD risk in this New Zealand group. This is despite their genetic background being comparable to that originally described by Rioux *et al.*<sup>(1)</sup>.

Crohn's disease subjects also completed a dietary questionnaire that characterised foods as alleviating symptoms, exacerbating symptoms or having no effect. The data from forty-four vegetable items were thus categorised, and the percentage of CD cases reporting benefits was plotted against the percentage showing adverse effects for each individual vegetable (Fig. 1). Two items stand out. Maize and mushrooms have high proportions reporting adverse effects, 49 and 39%, respectively, and low proportions reporting beneficial effects, 1.8 and 2.0% respectively.

It is noteworthy that ET occurs at higher levels in mushrooms than in other major items in a common diet<sup>(13)</sup>. These data led us to speculate that individuals carrying the variant *OCTN1* allele could show enhanced sensitivity to ET-containing foods, especially mushrooms. Fig. 2 shows the frequency of individuals carrying different *OCTN1* genotypes in relation to intolerance towards mushrooms. Although carrying a



**Fig. 1.** Percentage of Crohn's disease cases reporting ++ or + 'beneficial' responses compared with percentage reporting -- or - 'adverse' responses to forty-four vegetables. Key to vegetables in numeric order: maize; mushrooms; potato chips; leeks; fried potatoes; baked beans; chick peas; dried beans; green banana; onions; peas; celery; garlic; cucumber; raw tomatoes; tomato sauce; cabbage; gherkins; roasted potatoes; avocado; tomato puree; eggplant; silverbeet or spinach; broccoli or cauliflower; asparagus; salad greens; green beans; capsicum; cooked tomato; puha; taro; Chinese greens; Jerusalem artichokes; cassava; potatoes baked in skin; beetroot; lentils; watercress; parsnip; carrot; kumara; boiled potatoes; yams; pumpkin.



**Fig. 2.** Frequency of Crohn's disease cases with specific genotypes reporting adverse effects from mushrooms.

variant *OCTN1* gene does not adversely affect most individuals on a normal diet, this variant confers sensitivity to mushrooms in CD cases ( $P < 0.025$ ). Those individuals reporting mushroom intolerance show a strong bias towards carrying the variant allele. This statistically significant interaction was not seen for maize ( $P = 0.07$ ), which does not contain ET.

Since ET is an antioxidant, high levels of ET might lead to an antioxidant overload in red blood cells or epithelium, leading to pro-oxidant effects and/or imbalance in immune reaction. An alternative possibility might involve effects on pro-inflammatory cytokines or heat shock protein 70<sup>(14)</sup>.

We believe that this is the first diet–gene interaction described in CD. Although this dietary sensitivity may be known to symptomatic CD subjects, the data begin to position us towards utilising genotyping as a diagnostic tool upon which to base informed dietary advice. It may be possible to use nutrigenomics principles, in order to develop foods especially suited to this group of individuals.

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was obtained from all subjects/patients. I. P., C. H. and L. R. F. designed research; C. H. and I. P. performed research; R. B. G. and M. L. B. contributed human subjects and DNA samples; P. S. D. and A. M. contributed analytic tools; C. M. T. and D. Y. H. analysed data; L. R. F. wrote the paper. None of the authors had any conflict of interest.

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