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**VARIATION IN POPULATIONS OF ENTERAL
MICROFLORA IN PEOPLE WITH COELIAC
DISEASE FOLLOWING THE IMPLEMENTATION OF
A GLUTEN FREE DIET**

A thesis in partial fulfillment of the requirements for the degree of

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ABSTRACT

Coeliac disease (CD) is a disorder resulting from interactions between diet, genome and immunity. This research seeks to further our understanding of the pathology of CD in regard to its secondary effects on the diversity of enteral microflora via changes in immune tolerance. It proposes that enteral mucosal pro-inflammatory change in CD is associated with a decrease in microbial diversity whilst remission from inflammation may result in an increase in enteral microbial diversity that could contribute to the restoration of tolerance. The first study analyses whether remission from active CD is associated with change in generic enteral microbial diversity by assessing people at diagnosis and following their response to gluten exclusion. A comparison is made to people without CD consuming a 'normal diet'. DGGE profiling of faecal microflora in subjects with CD at diagnosis (confirmed by serology and by duodenal biopsy) and over three consecutive months on a gluten-free diet (GFD) was performed and profiles were compared with those of age and gender matched control subjects taken at monthly intervals. Diversity of faecal microflora (measured as Simpsons Index) was significantly lower in people with CD than in control subjects. It was possible to distinguish the profiles of coeliac subjects at diagnosis from those obtained after three months on a GFD but it was not possible to distinguish between the samples from control subjects taken at monthly intervals. The profiles of CD subjects after three months on a GFD were more dissimilar to those of the control subjects than those obtained prior to dietary treatment, chiefly on the basis of three bands that were not found in the faeces of any control subjects.

The second study analyses dietary intake to determine if a lack of nutrients at diagnosis (before institution of a GFD) and at monthly intervals for three consecutive months post diagnosis (on a GFD) exists, as it is known that CD is associated with nutrient deficiencies resulting from malabsorption due to intestinal inflammation and damage. Subjects completed a customised food questionnaire at each sampling period. Dietary intake was analysed using Foodworks Professional 2007. Significant differences were identified in gluten, starch and carbohydrate intake but not in other macronutrients. Contrary to established literature, these analyses identified few significant differences in micronutrient intake within coeliac subjects over time, however, significant differences were found in iron and sodium.

Keywords: Coeliac disease, enteral intestinal microflora, gluten avoidance, DGGE, nutrient aberrations

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A glossary of key terms is included as Appendix A to ensure clarity of terminology used throughout this paper. Definitions of words *italicised* and **bolded** can be found in the glossary.

List of Abbreviations used

ADCC	antibody cell-mediated cytotoxicity
AGA	antigliadin antibodies
AMPs	antimicrobial peptides
AN-PEP	prolyl-endopeptidase from <i>Aspergillus niger</i>
APCs	antigen presenting cells
BCR	B-cell receptor
BIR	baculovirus inhibitor repeat
BMD	bone mineral density
CAMs	cell adhesion molecules
CARD	caspase-activating and recruitment domain
CCL	chemokine
CCR	chemokine receptor
CD	coeliac disease
cDNA-AFLP	cDNA amplified restriction fragment length polymorphism
CLA	conjugated linoleic acids
CTLA-4	cytotoxic T-lymphocyte-associated antigen-4
DCs	dendritic cells
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribose nucleic acid
DS	Down syndrome
ECM	extracellular matrix
ELISA	enzyme linked immunosorbent assay
EMA	endomysial antibody
FAE	follicle associated epithelium
FCM	flow cytometry
FCM-FISH	combination of FISH with flow cytometry detection
FFQ	food frequency questionnaire
Fiaf	fasting-induced adipocyte factor
FISH	fluorescent <i>in situ</i> hybridisation
FOS	fructo-oligosaccharides
FSANZ	Food Standards Australia New Zealand
FVL	factor V Leiden
GALT	gut associated lymphoid tissue
GDP-fucose	nucleotide-activated fucose
GF	gluten-free
GFD	gluten-free diet
GI	gastrointestinal tract
GLN	glutamine
GOS	galacto-oligosaccharides
GP	guinea pig
HLA	human leukocyte antigen
HR	human recombinant
HU	human umbilical cord
IBD	inflammatory bowel disease

IBS	irritable bowel syndrome
ICAM	intercellular cell adhesion molecule
IDA	iron deficiency anaemia
IE	intestinal epithelium
IEC	intestinal epithelial cells
IELs	intraepithelial lymphocytes
IFN	interferon
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IKK	I κ B kinase
IL	interleukin
LA	linoleic acid
LPL	lipoprotein lipase
LPS	lipopolysaccharides
LRRs	leucine rich repeats
M-CELL	microfold cell
MDP	muramyl dipeptide
ME	monkey eosophagus
MHC	major histocompatibility class molecule
MLN	mesenteric lymph nodes
MMPs	metalloproteinases
MOH	Ministry of Health
mRNA	messenger ribosenucleic acid
MYO9B	myosin IXB gene
NBS	intermediary nucleotide-bonding site
NF- κ B	nuclear factor kappa B
NHL	non Hodgkins lymphoma
NK T-cells	natural killer T-cells
NKG2D	innate immune receptor
NODs	nucleotide-binding oligomerisation domain proteins
NPV	negaitve predictive value
NSPs	non-starch polysaccharides
PAMPs	pathogen-associated molecular patterns
PCR	polymerase chain reaction
PGN	peptidoglycans
pIgA	polymeric IgA
pIgR	polymeric Ig receptor
PPAR- γ	peroxisome-proliferator-activated receptor gamma
PPM	parts per million
PRRs	pathogen recognition receptors
PPV	positive predictive value
PRO	proline

qPCR	quantitative real time polymerase chain reaction
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SCFAs	short chain fatty acids
SDD	selective digestive tract decontamination
SER	serine
slgA	secretory immunoglobulin A
SIR	standardised incidence ratio
SMR	standardised mortality ratio
STAT6	signal transducer and activator of transcription-6
TCR	T-cell receptor
TGF	transforming growth factor
TGGE	temperature gradient gel electrophoresis
TIR	toll/interleukin receptor with an intracellular tail containing a conserved region
TIRAP	TIR adaptor protein
TLRs	toll-like receptor
TNF	tumour necrosis factor
TPOA	thyro-peroxidase antibody
t-RFLP	terminal restriction fragment length polymorphism
TS	Turner syndrome
tTg	tissue transglutaminase
TTGE	temporal temperature gradient electrophoresis
WS	Williams syndrome
ZO-1	zonulin-1

Abbreviations used in clinical study

CSI	Coeliac subject I
CST	Coeliac subject T
CSU	Coeliac subject U
CSW	Coeliac subject W
CSZ	Coeliac subject Z
CSAA	Coeliac subject AA
CSAB	Coeliac subject AB
CSAC	Coeliac subject AC
CSCGA	control subject A
CSCGB	control subject B
CSCGC	control subject C
CSCGD	control subject D
CSCGE	control subject E
CSCGF	control subject F
CSCGG	control subject G
CSCGH	control subject H

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