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ELDERMET: Enhancing gut health in older Irish people through an improved understanding of intestinal bacteria

Key external stakeholders:

Geriatricians, Food Industry, Medical Community

Practical implications for stakeholders:

- Knowledge on how the dietary strategies of the elderly can be improved, through manipulation of gut microbiota composition

Main results:

- ELDERMET is a landmark project investigating the association between gut microbiota, food and health in the elderly in Ireland. We established that habitual diet controls the population of gut bacteria, which in turn impact on health. Now that the connection between diet, gut bacteria and health has been established by ELDERMET, the knowledge gained is being used to try to influence the foods eaten by older people, and to also promote health by shaping the design of new foods and food ingredients produced specifically for the older age-group. Further practical outcomes such as improved quality of life and new microbiota-based diagnostics for illness and disease highlight the significance and potential impact of ELDERMET. These outcomes will maintain the Irish healthcare system and food industry at the forefront of scientific excellence for understanding diet-health interactions.

Opportunity / Benefit:

It is clear that the composition of the gut microbiota is tightly linked to diet. An opportunity exists for food producers is to use the information gained in the ELDERMENT project relating to microbial populations linked to health, and to develop food interventions to introduce, or promote the growth of beneficial bacteria in the human gut. The knowledge obtained from this project provides an opportunity for food manufacturers to promote health by shaping the design of new foods and food ingredients produced specifically for the older age-group. Additionally, the results of the ELDERMET project will inform the Medical and Diagnostic communities.

Collaborating Institutions:

Teagasc, University College Cork

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External collaborators:	Prof. Paul O'Toole, Ms. Mairead Coakley and the ELDERMET Team at UCC

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1. Project background:

The intestinal microbial flora has an important role in human health. As well as interacting with the diet, gut bacteria can also play more complex roles such as modulation of the immune system. In addition, the composition of the microbiota may be important for reducing gut infections. Changes in the microbiota over the life of the individual are accompanied by changes in multiple heath parameters, and there is intense activity at international level to understand this complex interaction.
To develop the potential of the diet for promoting intestinal health, knowledge of the baseline composition of the human gut microbiota is required. In this landmark study, cutting-edge sequencing technology is used to determine the composition of the faecal microbiota in elderly Irish subjects. This information provides the Irish Food Industry and Medical Community with an essential platform consisting of the bacterial composition of healthy elderly individuals, and how it may be positively modulated through nutritional and/or probiotic/prebiotic approaches.

2. Questions addressed by the project:

- Can we assess the composition of the faecal microbiota of elderly volunteers in the Irish population, using state-of-the-art molecular techniques?
- Can we correlate diversity, composition, and metabolic potential of the faecal microbial metagenome with health, diet and lifestyle indices that are a) likely to be influenced by the microbiota or b) to influence the microbiota?
- Can we develop recommendations for specific dietary ingredients, foodstuffs, functional foods and/or dietary supplements, which will improve the health of elderly consumers?
- Can we provide evidence-based recommendations for prospective studies to determine the molecular mechanisms for health improvements promoted by specific food ingredients that modulate components of the microbiota?

3. The experimental studies:

The range of data published from the ELDERMET project to date is broad-ranging and illustrates the intra-disciplinary nature of the research. High-throughput, culture-independent molecular technologies have elucidated microbial community structure at much higher resolution than was previously possible. Two such methods, pyrosequencing and a phylogenetic array were compared to evaluate microbial classification potential. Sequence comparisons of the V4 and V6 regions of the 16S ribosomal RNA gene, present in all prokaryotes, amplified using 454 FLX Pyrosequencing and HITChip hybridizations showed good correlation between the phylogenetic classifications, especially at lower-order ranks (phylum, class, order, and to a lesser extent, family). The Ribosomal Database Project (RDP)-classifier consistently assigned most V4 sequences from human intestinal samples down to genus level with good accuracy and speed. Results show that HITChip hybridizations and resulting community profiles correlated well with pyrosequencing-based compositions, especially for lower-order ranks, indicating high robustness of both approaches. This was the first study illustrating the deepest sequencing of individual gastrointestinal samples (Claesson et al. 2009, 2010).

The microbiota is in a state of flux during dynamic life periods such as older age (O'Toole & Claesson, 2010). The biological consequences of such change are still being explored. Pyrosequencing characterisation of the faecal microbiota in 161 ELDERMET subjects and 9 younger control subjects found a distinct difference in core microbiota composition to that of younger adults. The faecal microbiota of elderly subjects was characterized by unusual phylum proportions and extreme variability. In addition, temporal stability of the microbiota at three months was illustrated in 85% of subjects (Claesson et al. 2011).

Culture technologies have been the traditional method of microbiological exploration of the microbiota. The degree of correlation between measurements generated from culture-dependent microbiological techniques and from next generation sequencing technologies were explored in three groups of intestinal bacteria. Bifidobacterium sp., Lactobacillus sp. and Enterobacteriaceae were enumerated on selective media through culture-dependent techniques, and proportional representation of these bacteria in the microbiota was determined through sequencing technology. Correlation between the two methods implies that a single method is capable of profiling intestinal Bifidobacterium, Lactobacillus and Enterobacteriaceae populations. However, both methods have advantages that justify their use in tandem. This was the first, extensive study

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to compare bacterial counts from culture-dependent microbiological techniques and from next generation sequencing technologies (O'Sullivan et al. 2011).

Clostridium difficile is an important nosocomial pathogen associated particularly with diarrhoeal disease in elderly individuals in hospitals and long-term care facilities. Carriage rate was examined by culture as a function of faecal microbiota composition in elderly subjects recruited from the community, including outpatient, short-term respite, and long-term hospital stay subjects. The highest rate was found in those in short and long-term hospital care. The dominant 072 ribotype was carried by 43% (12/28) of subjects while the hypervirulent strain R027 (B1/NAP1/027) was isolated from 3 subjects (11%), 2 of whom displayed *C. difficile*-associated diarrhoea (CDAD) symptoms at the time of sampling. A marked reduction in microbial diversity at genus level was observed in patients who had been diagnosed with CDAD at the time of sampling and from whom C. difficile R027 was isolated (Rea et al. 2012)

Analysis of ELDERMET Food Frequency Data provided the opportunity to determine correlations between diet, distinct microbiota profiles, and clinical biomarkers of health and/or disease. A range of focused studies also investigated compliance with Irish food based dietary guidelines in elderly subjects across a range of health strata. From those residing in both community and institutional settings (rehabilitation wards/out-patients clinics), a high consumption of low nutrient density foods was prominent, with poor compliance to recommended intakes of other food groups including dairy, breads and cereals which may have negative health implications. Fruit and vegetable consumption by those in institutionalised care were low compared to current dietary recommendations. As a consequence, a large proportion of older Irish adults may be at risk of inadequate intakes of important vitamins and minerals (Power et al. 2011) in particular those of relevance for health promotion and brain-ageing, where gender differences in folate status have been associated with cognition (O'Connor et al. 2011).

At present, there is no information, based on the compelling role of the intestinal bacteria, upon which to develop foods for the promotion or maintenance of health in the elderly. Food products / ingredients / functional foods (pre- or probiotics) or supplements based on ELDERMET research findings could be used in the management of common health problems where links with the composition of the intestinal microbiota have been associated such as allergies, cancer, digestive disorders, obesity and its related conditions (Clemente et al. 2012). In addition, the recent discovery by ELDERMET scientists through unbiased correlation analysis of new microbiota associations with multiple clinical parameters (Claesson et al. 2012) will form the basis for the provision of strategies that modulate the microbiota to promote and maintain health in the elderly.

4. Main results:

- State of the art sequencing technologies determined the composition of the faecal microbiota of elderly volunteers in the Irish population.
- We correlated diversity, composition, and metabolic potential of the faecal microbial metagenome with health, diet and lifestyle indices.
- Results of the studies undertaken have led to the development of recommendations for specific dietary ingredients, foodstuffs, functional foods and/or dietary supplements that will improve the health of elderly consumers.
- We have provided evidence which will be used for the provision of strategies that modulate the microbiota to promote and maintain health in the elderly.

5. Opportunity/Benefit:

An opportunity exists for the Food Industry to develop new foods and food ingredients produced specifically for the older age-group.

- **Dissemination:**

Main publications:

- Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HMB, Coakley M,Laks B, O'Sullivan O,Fitzgerald G, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW (2012). 'Gut microbiota composition correlates with diet and health in the elderly'. *Nature.* 488(7410):178-84. http://www.nature.com/nature/journal/v488/n7410/full/nature11319.html
- Claesson, MJ, O. O'Sullivan , Q. Wang , J. Nikkilä, JR Marchesi, H. Smidt, W. M. de Vos, RP Ross, and PW O'Toole. (2009). 'Comparative Analysis of Pyrosequencing and a Phylogenetic Microarray for Exploring

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- Claesson and O' Toole PW (2010). 'Evaluating the latest high-throughput molecular techniques for the exploration of microbial gut communities'. *Gut microbes* (4):277-278.
- Claesson MJ Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G, Stanton C, van Sinderen D, O'Connor M, Harnedy N, O'Connor K, Henry C, O'Mahony D, Fitzgerald AP, Shanahan F, Twomey C, Hill C, Ross RP, O'Toole PW (2010). 'Composition, variability and temporal stability of the intestinal microbiota of the elderly'. *Proc Natl Acad Sci USA.* 15; 108 Suppl 1:4586-91.
- O'Connor E.M., Fitzgerald G.F, O' Toole P.W. (2011) 'Gender differences in folate status are associated with cognition in healthy Irish elderly adults'. *Proceedings of the Nutrition Society* (2011) 70 (OCE3) E64. DOI:10.1017/S0029665111001042
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- O'Toole P.W. & Claesson M.J. (2010) 'Gut microbiota: Changes throughout the lifespan from infancy to elderly' *International Dairy Journal* (2010) 20(4): 281-291.
- Power S.E., O'Toole P.W., O'Connor E.M., Fitzgerald G.F. (2011) 'Consumption of fruit and vegetables among elderly Irish people in long term institutionalised care: the ELDERMET project' *Proceedings of the Nutrition Society* 70 (OCE3) E116. DOI:10.1017/S002966511100156X
- Rea MC, O'Sullivan O, Shanahan F, O'Toole PW, Stanton C, Ross RP, Hill C (2012). 'Clostridium difficile carriage in elderly subjects and associated changes in the intestinal microbiota'. *J Clin Microbiol.*; 50(3):867-75.

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