Tailoring Foods to Match People’s Genes in New Zealand: Opportunities for Collaboration

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Personalized nutrition according to genotype is based on the premise that optimized dietary advice for one individual may not be appropriate for others, and that optimal health and wellbeing can be tailored according to genotype.

Nutrigenomics New Zealand is a collaboration between The University of Auckland and two Crown Research Institutes: Plant & Food Research and AgResearch \cite{1}. The program involves 55 named individuals, located at 5 different sites across New Zealand. In order to develop the appropriate methodologies and learn how to apply them, we are studying dietary response according to genotype in inflammatory bowel diseases (IBD), especially Crohn’s disease (CD), as proof of principle.

IBD are common gastrointestinal disorders, whose incidence appears to be rising in various countries, including New Zealand \cite{2}. Although the diseases are not invariably lethal, the symptoms, which include abdominal cramps and bloody diarrhea, can be debilitating and may result in poor nutrient intakes and severe malabsorption. While there is an apparent familial component to the disease, twin studies have confirmed that genetic variations influence disease susceptibility, rather than inevitably leading to the disease per se \cite{3}. The important observation that diet influences disease but that no single diet suits all \cite{4}, makes this an interesting candidate disease in which to study gene-diet interactions. An overview of the approach taken across the program is shown in figure 1.

Role of Genetics in CD in New Zealand

As with other genetic disorders, knowledge of key genes initially depended upon candidate gene studies, and it was not until 2001 that the first gene unequivocally
associated with CD, nucleotide oligomerization domain 2 (NOD2), was identified [5]. The accelerated progress afforded by genome association studies, using high-density arrays (SNP Chips) combined with large population groups and meta-analysis, has now associated more than 30 genes with susceptibility to CD [6]. We have generally not attempted to find novel genes, but confirmed overseas studies in our

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**Fig. 1.** Outline of the approach used to develop personalized foods, tailored to genotype, by Nutrigenomics New Zealand. Genes associated with specific disease are identified from genetic epidemiology studies, particularly genome-wide association studies (GWAS), and confirmed for importance and relevance to the New Zealand population. The phenotypic effects of those genes which appear particularly important in this country are mimicked in a cell-based reporter gene assay, which is used for testing a wide range of food components and/or food extracts as a high throughput assay. Foods for preferential testing in that assay are identified through matching dietary tolerances or intolerances to specific genotypes (see fig. 2). Those foods that appear to show ability to restore the wild-type phenotype in the mutant reporter gene assay and/or show strong links to genotype from the dietary questionnaires are then tested in animal studies. A systems biology approach in specific animal models is then used to understand how different foods or food compounds might interact with a particular genotype. One output of this approach is a defined set of biomarkers for use in human trials. Human studies utilize subjects stratified according to genotype. Study participants are randomized to a control diet or an experimental diet that includes the food shown to ameliorate the phenotype associated with a particular genotype in animal models. Biomarkers previously identified in those animal studies are monitored to rapidly establish if the experimental diet can restore the non-risk-genotype profile, without the need to follow study participants until the development of disease. Positive results in these studies are considered to provide preliminary evidence to validate the use of that food in subjects carrying the variant genotype.
New Zealand population group. Examples of key genes in this country are given in table 1 [7–12]. Most of the genes appear to affect immune response and/or bacterial recognition [13]. It is noteworthy that not all genes relevant in other countries are necessarily key genes for susceptibility to this disease in New Zealand, as might be expected [14]. It is also becoming increasingly apparent that risk may associate with gene-gene interactions, rather than a single gene per se [15].

### Table 1. Examples of genes associated with CD in a Caucasian population from New Zealand

<table>
<thead>
<tr>
<th>Gene</th>
<th>Abbreviation</th>
<th>SNP</th>
<th>Allelic odds ratio, CD vs. control</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila discs large homolog 5</td>
<td>DLG5</td>
<td>rs1248696</td>
<td>1.29 (0.93, 1.78)</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2289310</td>
<td>0.90 (0.48, 1.66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2289311</td>
<td>0.83 (0.67, 1.03)</td>
<td></td>
</tr>
<tr>
<td>Nucleotide-binding oligomerization domain containing 1</td>
<td>NOD1</td>
<td>rs2075818</td>
<td>0.66 (0.49–0.89)</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2075822</td>
<td>0.91 (0.69–1.21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2907748</td>
<td>0.84 (0.64–1.10)</td>
<td></td>
</tr>
<tr>
<td>Nucleotide-binding oligomerization domain containing 2</td>
<td>NOD2</td>
<td>rs2066844</td>
<td>2.7 (1.5–5.2)</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2066845</td>
<td>2.4 (0.94–6.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2066847</td>
<td>4.4 (1.6–12)</td>
<td></td>
</tr>
<tr>
<td>Toll-like receptor 4</td>
<td>TLR4</td>
<td>rs4986790</td>
<td>1.225 (0.79, 1.91)</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4986791</td>
<td>1.046 (0.67, 1.63)</td>
<td></td>
</tr>
<tr>
<td>Tumour necrosis factor alpha</td>
<td>TNF-alpha</td>
<td>rs1800629</td>
<td>1.10 (0.72–1.68)</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1799724</td>
<td>1.09 (0.84–1.42)</td>
<td></td>
</tr>
<tr>
<td>Tumour necrosis factor receptor superfamily, member 1B</td>
<td>TNFRSF1B</td>
<td>rs1061622</td>
<td>1.12 (0.87–1.45)</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1061624</td>
<td>0.98 (0.79–1.22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3397</td>
<td>0.91 (0.73–1.13)</td>
<td></td>
</tr>
</tbody>
</table>

**Modeling Genetic Variation in Human CD Populations in vitro**

Once a key genotype is established as being important, high throughput screens are developed to test whether selected food components can overcome the phenotype of the functional SNP. For example, Philpott et al. [16] described a reporter gene assay to test effects of foods on the common NOD2 variant, while Danesi et al. [17] have established a screen for IL23R. Robotic systems using 384-well plates enable high numbers of food components to be tested in a given experiment. These then provide leads which may be confirmed by dietary analyses and which can be more extensively studied in animal models.
### Fig. 2.
Example of a worksheet to identify gene-diet interactions in CD in the New Zealand population. For individuals carrying each of the genetic variants (identified as per Fig. 1), we considered self-diagnosed tolerance, neutral effects or intolerance to 269 individual dietary items. We have calculated the probability that each individual food significantly improves or worsens the condition, as previously described [14]. A matrix is then created linking genotype to food tolerance. A section of such a matrix is illustrated.

### Estimating the Role of Diet in CD

There is not complete agreement as to what dietary assessment methods are appropriate in epidemiologic studies, and the same is true of nutrigenetic studies. Inevitably there needs to be a compromise between the most accurate record possible and what is practically acceptable to the study population. In particular, many food-frequency questionnaires cluster similar dietary items in order to retain people's attention and not make recording too large a burden. However, in our CD population, we elected to use a much larger list of food items, so that subtle differences in potentially bioactive
food components, rather than broad classes of nutrients could be tested [14]. An example of part of the dietary information becoming available for CD is given in figure 2. It can be seen that the same food that is beneficial to one individual may actually trigger symptoms of disease in others.

In the specific case of CD, it is important to realize that food preparation methods may be as important as food components per se. For example, many of our subjects reported that they could tolerate tomatoes, but only when they are peeled and seeded. Ginger ale was typically detrimental, but could be beneficial when allowed to go flat. Kiwifruit eaten as a whole fruit was often detrimental, but a commercially available juice in which the seeds are filtered out could be beneficial. So, knowledge of preparation details may be as important as knowledge of the food items themselves.

Animal Models of IBD

Patients with IBD are a highly sensitive population and it is essential that any potential nutritional therapies are rigorously tested in animal models for in vivo effects before considering human clinical trials. Nutrigenomics New Zealand has utilized 2 different models: the multidrug resistant mouse, and the interleukin-10 knockout mouse [18–23]. A 2 × 2 study design considers the wild-type mouse versus the relevant knockout, in the presence or absence of the dietary item to be tested. The experiments are run for sufficient time for disease (or lack thereof) to be established, the animals euthanized and tissues culled for various endpoints. These range from pathologic assessment of disease presence/absence/severity, to transcriptomics (microarrays), proteomics and/or metabolomics techniques [18–23].

Double-Blind Placebo Controlled Human Clinical Trials

Ultimately, the proof of efficacy of a dietary component in a given genotype or population group depends upon a randomized human clinical trial. Ethical constraints make time to disease an inappropriate endpoint, and biomarkers (or surrogate disease endpoints) become essential. These rely upon collection of a readily accessible tissue (blood, urine, buccal swab or feces) before and after a given period of a defined dietary intervention in a genetically stratified population [24].

There are few published studies of gene-specific approaches to clinical trials. However, Kornman et al. [25] tested effects of their proprietary botanical mixture on inflammation, using C-reactive protein as an endpoint. They showed that the level of C-reactive protein was reduced in the intervention arm of their study, and suggested their preparation would have beneficial effects on inflammation in human populations. This would be likely to relate to chronic human diseases such as cardiovascular disease, but may also be highly relevant to inflammatory disorders such as IBD.
Data Management and Integration

Integral to this study approach are very large datasets. These need to be maintained with confidentiality, but must also have the ability to be interrogated by different individuals with different expertise, working in different locations. In Nutrigenomics New Zealand, we maintain an interdisciplinary wiki that enables cross-disciplinary communication, and enables the analysis of complex multidimensional interactions. Database management, bioinformatics and biostatistics are essential tools whose importance must not be underestimated in a major program of this sort. A relational database may help to reveal relationships among genetic variation, dietary patterns and disease states. A significant analytical challenge remains in reducing the dimensionality of such complex datasets.

The most significant challenge, however, is also common to other nutrigenomics programs. That is, the recognition that adequately powered studies require literally thousands of individuals. This provides numerous opportunities, indeed reflects a necessity, for strategic international alliances for collaboration [26].

Acknowledgments

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References

1. www.nutrigenomics.org.nz


