

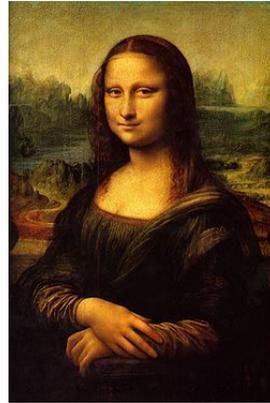
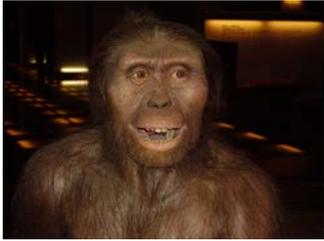
Brief overview of research activities at Beltsville Human Nutrition Research Center (BHNRC)

Thomas Wang, Ph.D. Research Leader, Diet
Genomic and Immunology Laboratory (DGIL)

In Memory of Dr. John A. Milner,
BHNRC Center Director.(1947-2013)



Evolution of Nutrition Research



Observational
“What my
grandma said”

Medicine



Biochemistry
Molecular biology
Immunology

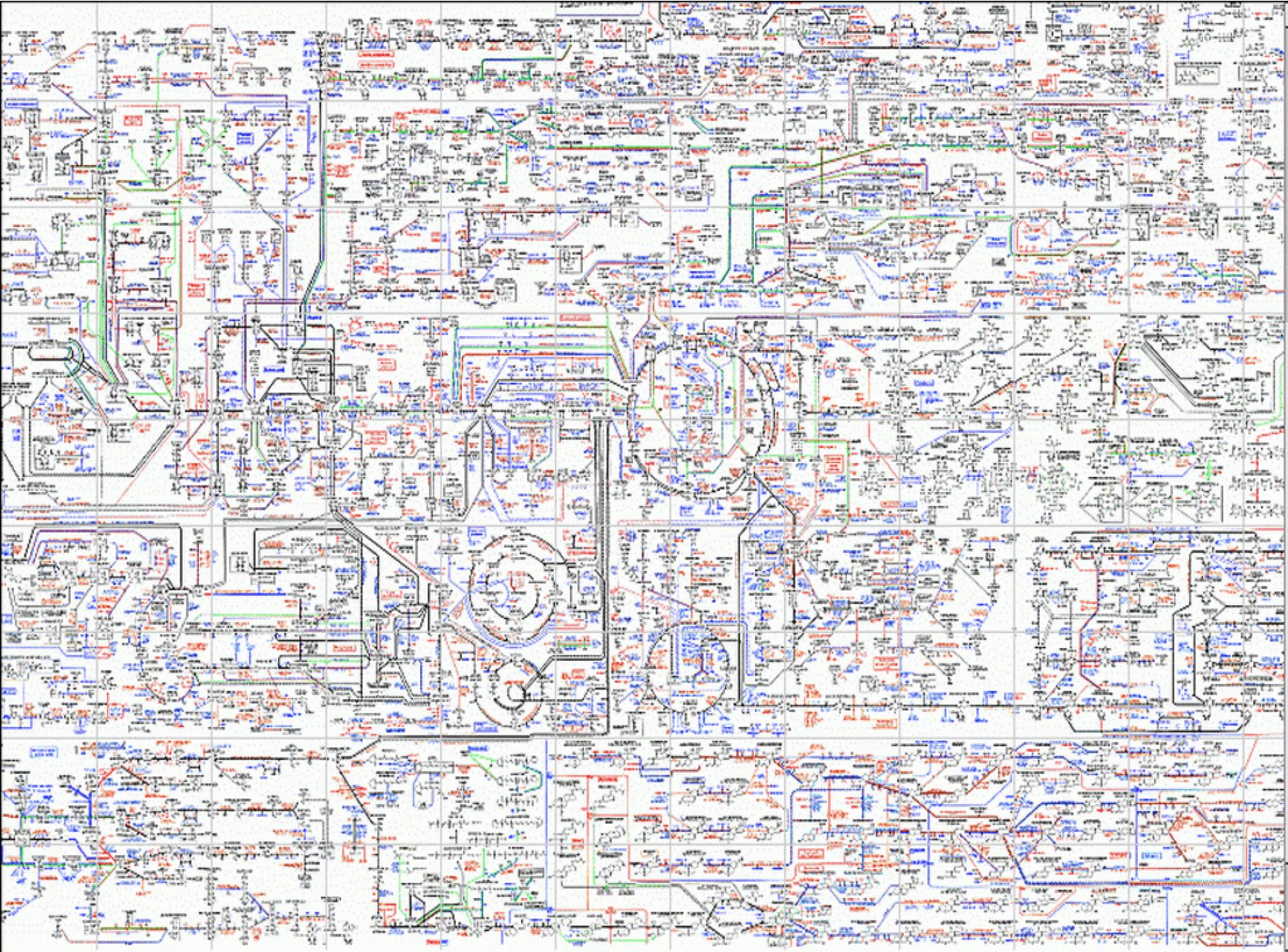


Nutrigenomics
Nutrigenetics
Diet-gene interaction

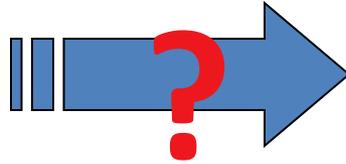
Prevention of
deficiency



**Prevention of
chronic diseases:
obesity diabetes,
cancer**



The questions to be address



**Promote health and
decreases risk of chronic
diseases such as cancer**

- Jury's still out on beneficial effects of fruit and vegetable on cancer prevention. Complexity of food matrix. Cancer is not one disease.
- Active compound(s) and interaction between compounds unclear (phytochemicals).
- Genetic background such as differences in xenobiotic enzymes may influence effects of phytochemicals and vice-versa.
- Molecular targets of phytochemicals in cell and their mechanisms of action remain unclear.
- In vitro or animal study may not equal human effects.
- Natural compounds are consider safe and may be use for chemoprevention. The issue of supplement.



Wilbur Olin Atwater (1844-1907) considered the father of modern nutrition research and education, was the U.S. Department of Agriculture's first chief of nutrition investigations.

“After completing his study, Atwater concluded that Americans consumed too much fat and sweets and did not exercise enough.” (wikipedia)

Beltsville Human Nutrition Research Center (BHNRC)

The Beltsville Human Nutrition Research Center (BHNRC) is the oldest and most comprehensive of six human nutrition research centers within the Agricultural Research Service. The largest of USDA's human nutrition research facilities and the home of the first human nutrition research conducted by USDA, dating back to the late 1890's.

The mission of BHNRC is to define, through research, the role of food and its components in optimizing human health and in reducing the risk of nutritionally related disorders in the diverse population.

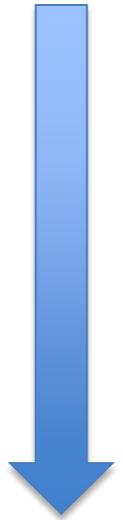
Impact

Our research (science-based information) is important to policy-makers, food producers, educators, other scientists and consumers in better understanding the relationships between agriculture, diet, and health.

BHNRC Laboratories

Diet, Genomics, and Immunology Laboratory
Food Components and Health Laboratory
Food Composition and Methods Development Laboratory
Food Surveys Research Group
Nutrient Data Laboratory

Basic



Apply

Nutrient Data Laboratory (NDL)

Pamela Pehrsson, Research Leader
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NDL is responsible for developing and maintaining the USDA National Nutrient Database, a database of over 140 nutrient and food components contained in over 7,000 foods in the US food supply.

Examples of NDL work:

USDA National Nutrient Database for Standard Reference

Other Databases and Reports

Dietary Supplement Ingredient Database, Release 2 (DSID-2)

Nutritive Value of Foods (Home and Garden Bulletin No. 72). Revised October 2002.

Cooking Yield and Nutrient Retention Factors

Special Interest Databases:

Choline, Release 2 (2008)

Flavonoids, Release 3.1 (December 2013)

Fluoride, Release 2 (2005)

Isoflavones, Release 2 (2008)

Oxalic Acid Content of Selected Vegetables

Proanthocyanidins

Key Foods

18970, Tortillas, ready-to-bake or -fry, flour, shelf stable

Food Group: Baked Products

Nitrogen to Protein Conversion Factor: 6.25

Nutrient values and weights are for edible portion

Nutrient	Unit	Value per 100 g	# of Data Points	Std. Error	tortilla 49g	package 484g
Proximates						
Water ¹	g	32.43	12	0.502	15.89	156.96
Energy	kcal	297	--	--	146	1437
Energy	kJ	1244	--	--	610	6021
Protein ¹	g	8.01	12	0.189	3.92	38.77
Total lipid (fat) ¹	g	7.58	12	0.336	3.71	36.69
Ash ¹	g	2.71	12	0.115	1.33	13.12
Carbohydrate, by difference	g	49.27	--	--	24.14	238.47
Fiber, total dietary ¹	g	2.4	12	0.108	1.2	11.6
Sugars, total ¹	g	2.66	12	0.151	1.30	12.87
Sucrose ¹	g	0.48	12	0.193	0.24	2.32
Glucose (dextrose) ¹	g	0.09	12	0.050	0.04	0.44
Fructose ¹	g	0.03	12	0.030	0.01	0.15
Maltose ¹	g	2.06	12	0.086	1.01	9.97

Other Nutrients

Starch

Minerals (11)

Vitamins (30)

Amino Acids

Fatty Acids

Cholesterol

Caffeine

Theobromine

Alcohol

Profiles by serving size

www.ars.usda.gov/nutrientdata

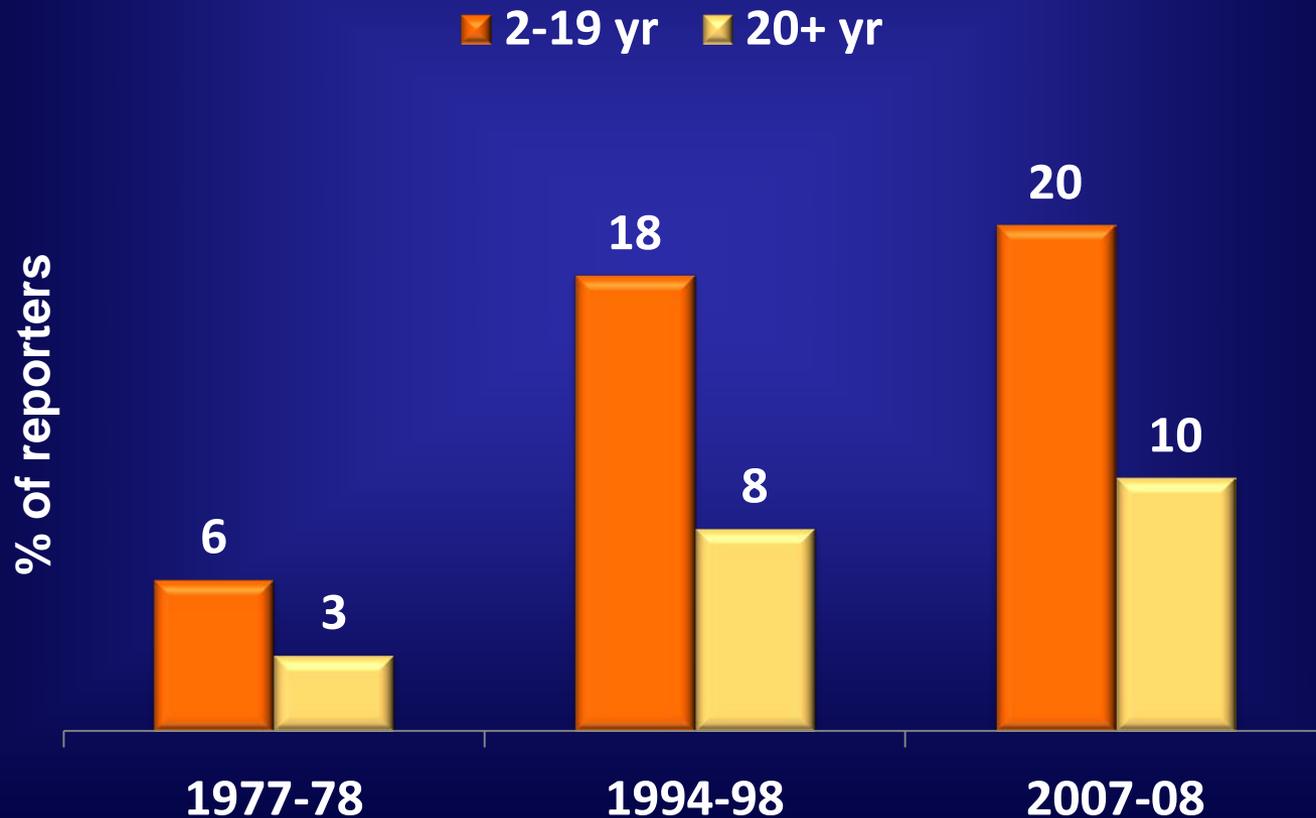
Food Surveys Research Group (FSRG)

Alanna Moshfeqh, Research Leader

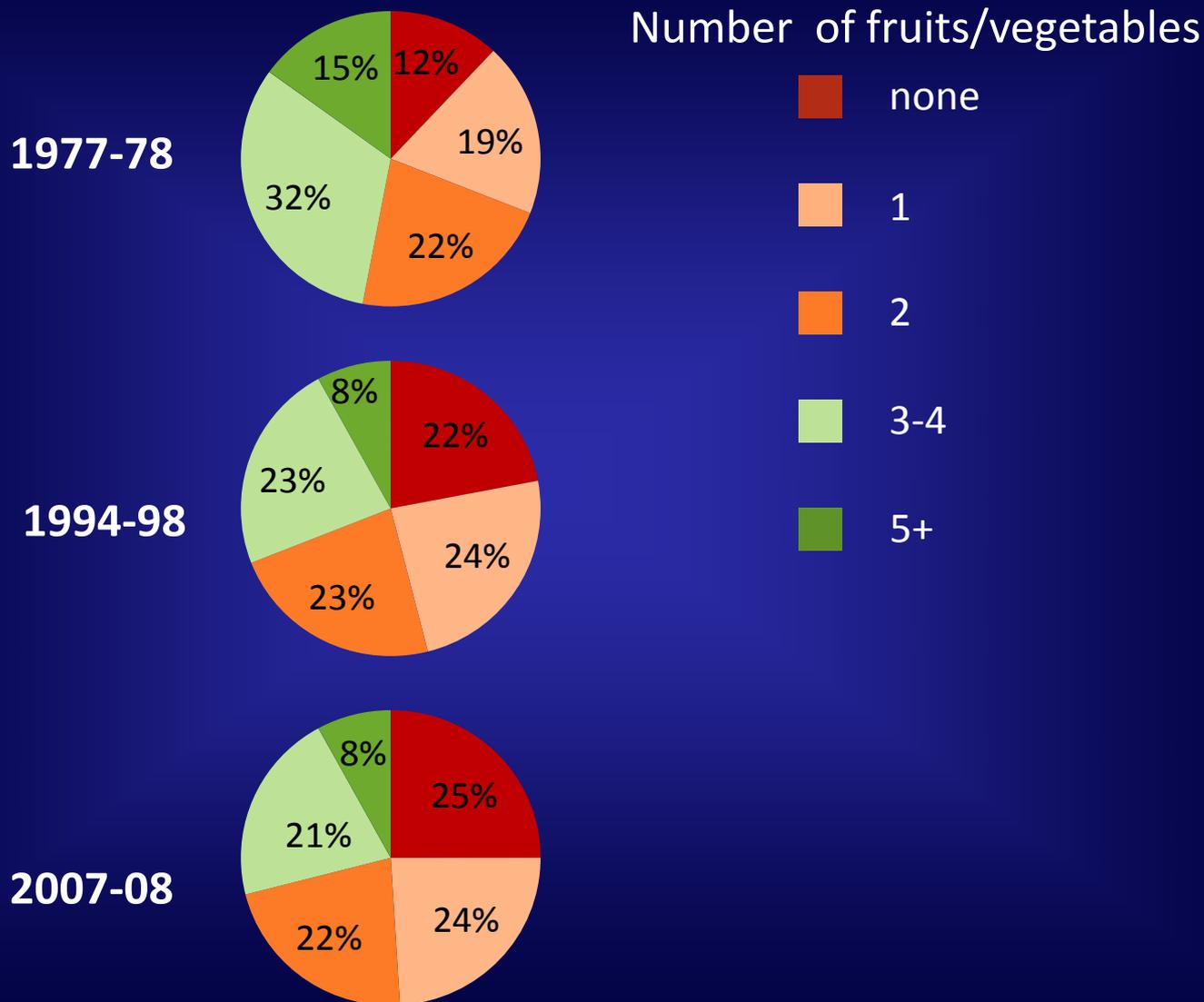
Alanna.Moshfeqh@ars.usda.gov, 301-504-0170

FSRG is responsible for “What We Eat in America”, the dietary assessment component of the National Health and Nutrition Examination Survey (NHNES), conducted annually by the National Center for Health Statistics.

Change in Pizza Consumption for individuals reporting pizza



Change in Percent Reporting Fruits/Vegetables



Source: 1977-78 NFCS, 1994-98 CSFII, 2007-08 WWEIA, NHANES, 1-day, individuals 2yrs+, excluding breast-fed children

Food Composition and Methods Development Laboratory (FCMDL)

James Harnly, Research Leader

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FCMDL develops innovative measurement systems for the determination of food components that influence human health.

Goals

Design and develop improved analytical methodology for the determination of food components.

Transfer developed analytical technologies to scientific communities in the US and worldwide.

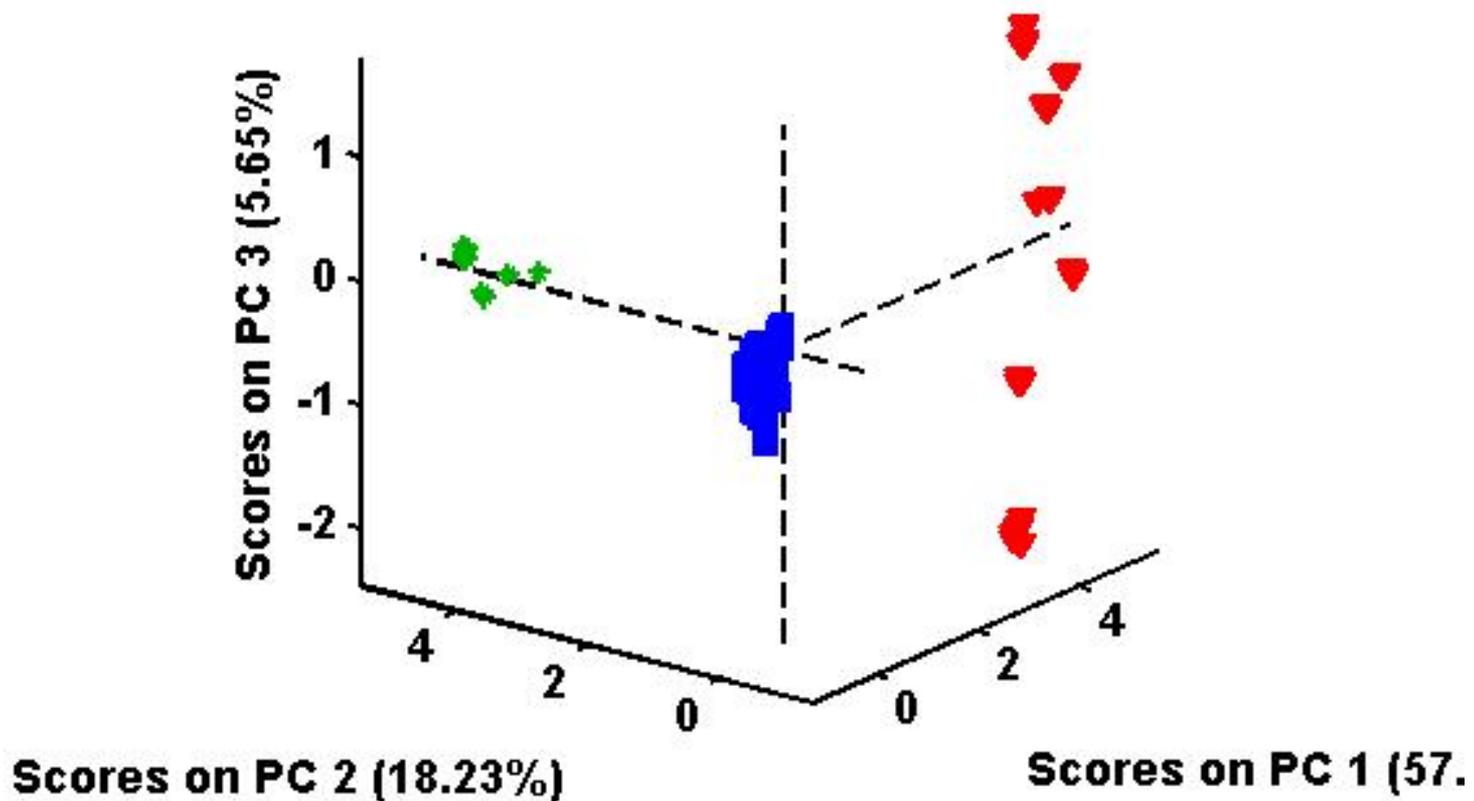
To improve the quality of food composition data and databases.

Differentiation of Panax species using principal component analysis and high resolution mass spectrometry

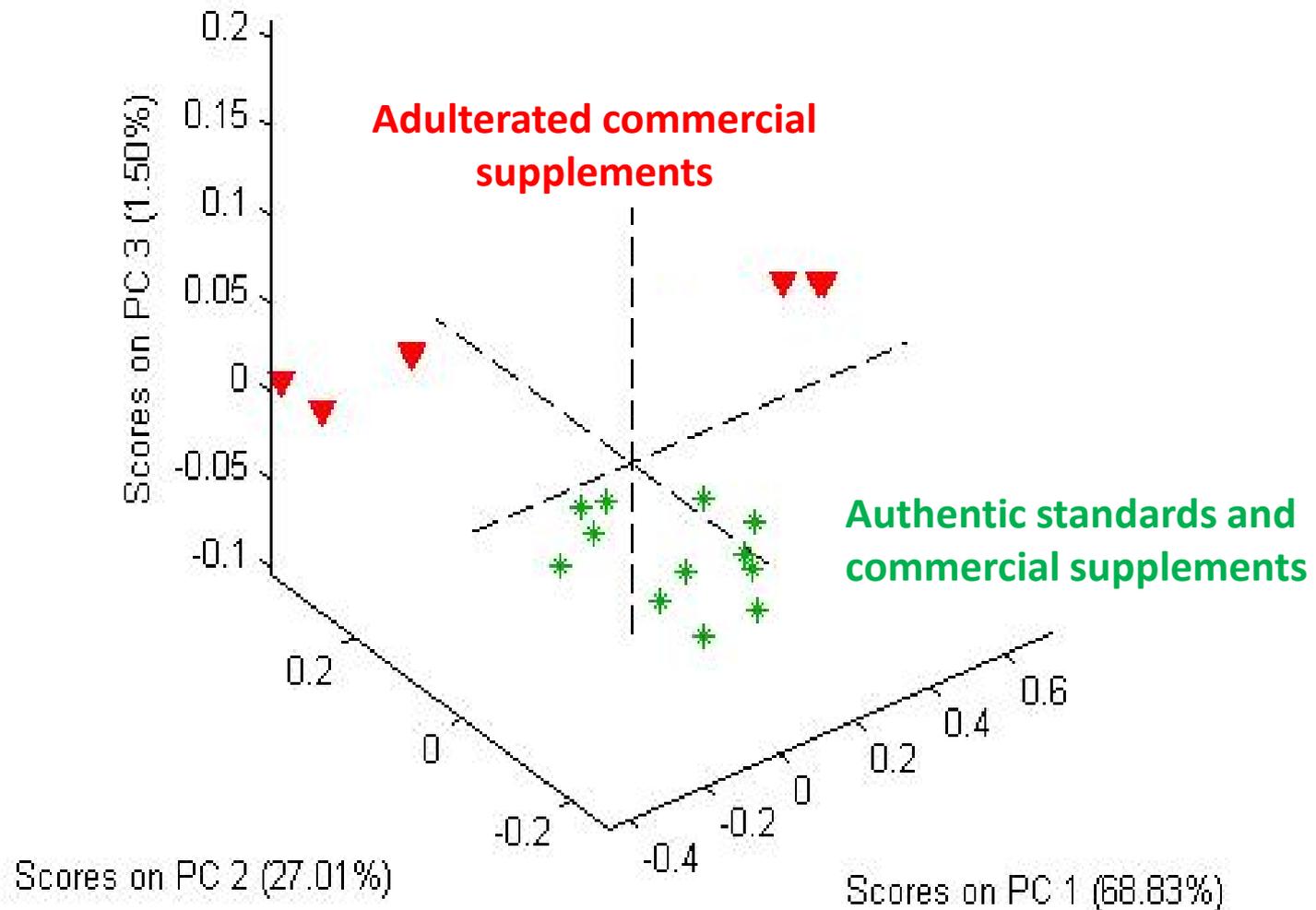
Asian ginseng of different ages (*Panax ginseng*)

American ginseng from Wisconsin (*P. quinquefolius*)

Notoginseng (*P. notoginseng*)



Detection of adulterated *Ginkgo biloba* supplements using principal component analysis and high resolution mass spectrometry



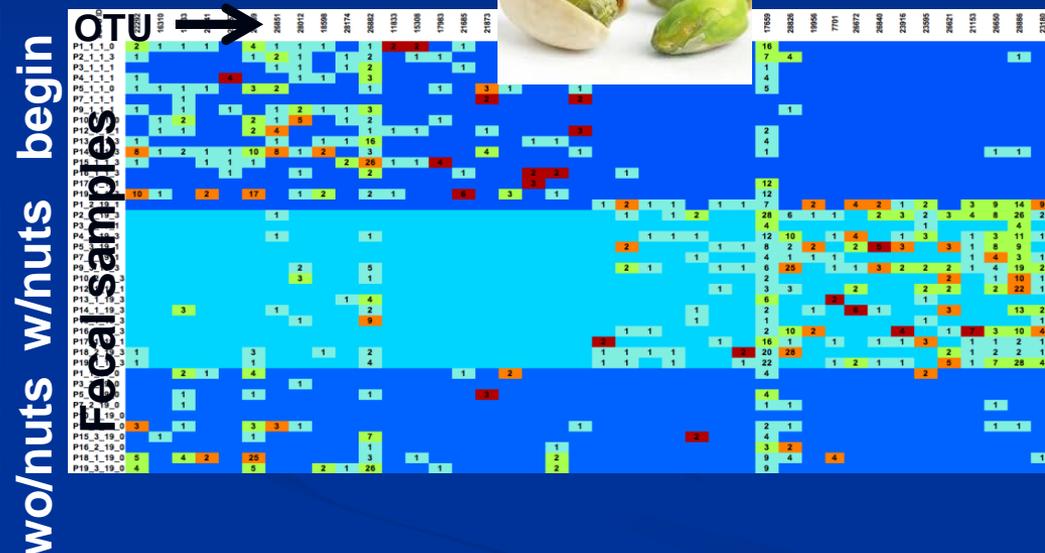
Food Components and Health Laboratory (FCHL)

David Baer, Research Leader

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FCHL conducts research on metabolic/physiologic responses of individuals to whole foods and dietary constituents including traditional nutrients, phytochemicals, and functional foods.

Heat Map of Bacterial OTUs Affected by Nut Consumption



- Both nuts affected various OTUs - the specific effects differed.
 - Only *Clostridium_sp._ASF356* and *Firmicutes_bacterium_DJF_VP44* were both decreased by consumption of both nuts.
- Stronger effect was observed for pistachios.

Diet, Genomics, and Immunology Laboratory (DGIL)

Thomas Wang, Research Leader,
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DGIL conducts research on metabolic/physiologic responses to dietary intervention focusing on nutrients, chronic disease, immune function, probiotics, and genetic expression in animal models and humans.

The mission of the Diet, Genomics, and Immunology Laboratory (DGIL) focuses on these main areas: (1) determine the basic mechanism of action of nutrients; (2) identify chemical forms and bioavailability of nutrients in foods; (3) develop cellular, molecular and immunological methods for evaluating nutrient bioavailability, metabolic function, requirements, and their effects on immune function and inflammation.

Two databases are maintained by DGIL:

- Porcine Translational Research Database
- Phytochemical Database (Collaborate with NDL)

Analyses of pig genomes provide insight into porcine demography and evolution

A list of authors and their affiliations appears at the end of the paper

For 10,000 years pigs and humans have shared a close and complex relationship. From domestication to modern breeding practices, humans have shaped the genomes of domestic pigs. Here we present the assembly and analysis of the genome sequence of a female domestic Duroc pig (*Sus scrofa*) and a comparison with the genomes of wild and domestic pigs from Europe and Asia. Wild pigs emerged in South East Asia and subsequently spread across Eurasia. Our results reveal a deep phylogenetic split between European and Asian wild boars ~1 million years ago, and a selective sweep analysis indicates selection on genes involved in RNA processing and regulation. Genes associated with immune response and olfaction exhibit fast evolution. Pigs have the largest repertoire of functional olfactory receptor genes, reflecting the importance of smell in this scavenging animal. The pig genome sequence provides an important resource for further improvements of this important livestock species, and our identification of many putative disease-causing variants extends the potential of the pig as a biomedical model.

The domestic pig (*Sus scrofa*) is a eutherian mammal and a member of the Cetartiodactyla order, a clade distinct from rodent and primates, that last shared a common ancestor with humans between 79 and 97 million years (Myr) ago^{1,2} (<http://www.timetree.net>). Molecular genetic evidence indicates that *Sus scrofa* emerged in South East Asia during the climatic fluctuations of the early Pliocene 5.3–3.5 Myr ago. Then, beginning ~10,000 years ago, pigs were domesticated in multiple locations across Eurasia³ (Frantz, L. A. F. *et al.*, manuscript submitted).

Here we provide a high-quality draft pig genome sequence developed under the auspices of the Swine Genome Sequencing Consortium^{4,5}, established using bacterial artificial chromosome (BAC)⁶ and whole-genome shotgun (WGS) sequences (see Methods and Supplementary Information). The assembly (Sscrofa10.2) comprises 2.60 gigabases (Gb) assigned to chromosomes with a further 212 megabases (Mb) in unplaced scaffolds (Table 1 and Supplementary Tables 1–3).

Genome annotation

A *de novo* repeat discovery and annotation strategy (Supplementary Fig. 8) revealed a total of 95 novel repeat families, including: 5 long interspersed elements (LINEs), 6 short interspersed elements (SINEs), 8 satellites and 76 long terminal repeats (LTRs). The relative content of repetitive elements (~40%, Supplementary Figs 9 and 10) is lower than reported for other mammalian genomes. The main repetitive element groups are the LINE1 and glutamic acid transfer RNA (tRNA^{Glu})-derived SINEs or PRE (porcine repetitive element). The expansion of PRE is specific to the porcine lineage. Phylogenetic analysis of LINE1 and PRE (Supplementary Figs 13 and 14) indicates that only a single lineage of each is currently active and that the main expansion of both LINE1 and PRE occurred in the first half of the Tertiary period. Smaller expansions, particularly in LINE1, have occurred since, but recent activity is very low (Supplementary Information).

Annotation of genes, transcripts and predictions of orthologues and paralogues was performed using the Ensembl analysis pipeline⁷ (Table 1 and Supplementary Figs 3–7). Further annotation for non-protein-coding RNAs (ncRNAs) was undertaken with another analysis pipeline (Supplementary Information and Supplementary Table 4).

Evolution of the porcine genome Evolution of genes and gene families

To examine the mutation rate and type of protein-coding genes that show accelerated evolution in pigs, we identified ~9,000 as 1:1 orthologues within a group of six mammals (human, mouse, dog, horse, cow and pig). This orthologous gene set was used to identify proteins that show accelerated evolution in each of these six mammalian lineages (Supplementary Information). The observed number of synonymous substitutions per synonymous site (dS) for the pig lineage (0.160) is similar to that of the other mammals (0.138–0.201) except for the mouse (0.458), indicating similar evolutionary rates in pigs and other mammals. The observed dN/dS ratio (ratio of the rate of non-synonymous substitutions to the rate of synonymous substitutions) of 0.144 is between those of humans (0.163) and mice (0.116), indicating an intermediate level of purifying selection pressure in the pig. Genes showing increased dN/dS ratios in each lineage were analysed using DAVID⁸ to examine whether these rapidly evolving genes were enriched for specific biological processes. Most lineages show different fast-evolving pathways, but some pathways are shared (Fig. 1).

Immune genes are known to be actively evolving in mammals^{9,10}. Because many immune genes were not included in the analysis of 1:1 orthologues, we examined a randomly selected subset of 158 immunity-related pig proteins for evidence of accelerated evolution (Supplementary information). Twenty-seven of these genes (17%)

Table 1 | Assembly and annotation statistics

Assembly	Placed	Unplaced	Annotation*
Total length	2,596,639,456	211,869,922	21,640 protein-coding genes
Unmapped length	2,323,671,356	195,490,322	380 pseudogenes
Scaffolds	5,343	4,562	2,965 ncRNAs†
Contigs	73,524	168,358	197,675 gene exons
Scaffold N50	637,332	98,022	26,487 gene transcripts
Contig N50	80,720	2,423	

* Numbers refer to the annotation performed by Ensembl (release 67). Results of an independent annotation by the NCBI can be obtained from <http://www.ncbi.nlm.nih.gov/mapview/stats/BuidStats.cgi?taxid=9823&build=4&ver=1>.

† An improved ncRNA annotation with 3,601 ncRNAs and structured elements is available as a separate track in Ensembl version 70 and for download from <http://rth.dkl/resources/annnotator/susscr102.N50>. 50% of the genome is in fragments of this length or longer.

Detection of *Bifidobacterium animalis* subsp. *lactis* (Bb12) in the Intestine after Feeding of Sows and Their Piglets[†]

Gloria Solano-Aguilar,^{1*} Harry Dawson,¹ Marta Restrepo,¹ Kate Andrews,²
Bryan Vinyard,³ and Joseph F. Urban, Jr.¹

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Received 5 February 2008/Accepted 4 August 2008

A real-time PCR method has been developed to distinguish *Bifidobacterium animalis* subspecies in the gastrointestinal tracts of pigs. Identification of a highly conserved single-copy *tuf* gene encoding the elongation factor Tu involved in bacterial protein biosynthesis was used as a marker to differentiate homologous *Bifidobacterium animalis* subsp. *lactis* (strain Bb12) from *Bifidobacterium animalis* subsp. *animalis*, as well as *Bifidobacterium suis*, *Bifidobacterium breve*, *Bifidobacterium longum*, several species of *Lactobacillus*, and *Enterococcus faecium*. Real-time PCR detection of serially diluted DNA extracted from a pure culture of Bb12 was linear for bacterial numbers ranging from 10 to 10,000 *tuf* gene copies per PCR ($r^2 = 0.99$). Relative differences in Bb12 bacterial numbers in pigs fed daily with Bb12 were determined after detection of Bb12 *tuf* gene copies in DNA extracted from the intestinal contents. Piglets treated with Bb12 immediately after birth maintained a high level of Bb12 in their large intestines with continuous daily administration of Bb12. Piglets born to Bb12-treated sows during the last third of their gestation and also treated with Bb12 at birth (T/T group) had a higher number of Bb12 organisms per gram of intestinal contents compared to placebo-treated piglets born to placebo-treated sows (C/C group), Bb12-treated sows (T/C group), or piglets born to placebo sows but treated with Bb12 immediately after birth (C/T group). In addition, there was a significant increase in gene expression for Toll-like receptor 9 (TLR9) in piglets from the T/T group, with no change in TLR2 and TLR4. These findings suggest that the *tuf* gene represents a specific and functional marker for detecting *Bifidobacterium animalis* subsp. *lactis* strain Bb12 within the microbiota of the intestine.

Bifidobacteria are anaerobic, gram-positive, non-spore-forming, non-motile bacilli commonly found in the gastrointestinal tracts (GITs) of animals, including humans (1). Bifidobacteria are the predominant bacterial species in the GITs of infants; they represent about 3% of the total microbiota in the intestine of healthy adult humans (16) and are associated with beneficial health effects (15, 16, 30, 32, 36). Despite the general acceptance of bifidobacteria as a probiotic, and their use in health-promoting foods such as fermented milks, infant formula, cheese, and ice cream, there is little definitive information to support a mechanism of action. Stimulation of host resistance, immune modulation, and competitive exclusion of pathogens, however, have been proposed as likely mechanisms (40).

One of the *Bifidobacterium* species commonly used in the food industry is *Bifidobacterium animalis* subsp. *lactis* strain Bb12, which is marketed around the world under a variety of labels in dairy products and infant formulas (37, 40). The taxonomy of *B. animalis* subsp. *lactis* has been controversial since its original description by Meile et al. in 1997 (27), and

several studies have investigated its similarity with the closely related species *Bifidobacterium animalis* subsp. *animalis* (41). New genotypic evidence reported by Ventura et al. (46–48), Zhu and Dong (54), Masco et al. (23), and Kwon et al. (19) indicate that *B. animalis* subsp. *lactis* and *B. animalis* subsp. *animalis* should be considered two separate taxonomic entities at the subspecies level. *B. animalis* subsp. *lactis* exhibits properties such as elevated oxygen tolerance (34), differential growth in milk-based media (46), and hydrolysis of milk proteins (13); these properties differ from *B. animalis* subsp. *animalis* and facilitate its growth in commercial products under nonanaerobic conditions. Traditional bacteriological and biochemical identification techniques, such as selective growth of species in differential media, cannot be routinely used to differentiate *Bifidobacterium* species. These methods are time consuming and limited by low sensitivity and reproducibility due to the multitudes of species that grow and require further identification (24). In addition, the information obtained by culture-based growth methods provides only a fragmented picture of the relative distribution of species within the GIT because a significant part of its microbiota cannot be grown in vitro (43, 55). Culture-independent methods have been developed in recent years as an alternative to characterize whole bacterial communities by direct extraction of DNA from fecal samples without prior cultivation (6). These methods include fluorescent in situ hybridization, dot blot hybridizations, and DNA arrays and fingerprinting methods such as terminal restriction fragment length polymorphism and denaturing or

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[†] Published ahead of print on 8 August 2008.

Research Article

Indole-3-Carbinol and 3',3'-Diindolylmethane Modulate Androgen's Effect on C-C Chemokine Ligand 2 and Monocyte Attraction to Prostate Cancer CellsEun-Kyung Kim^{1,2,3}, Young S. Kim¹, John A. Milner^{1,4}, and Thomas T.Y. Wang³**Abstract**

Inflammation has a role in prostate tumorigenesis. Recruitment of inflammatory monocytes to the tumor site is mediated by C-C chemokine ligand 2 (CCL2) through binding to its receptor CCR2. We hypothesized that androgen could modulate CCL2 expression in hormone-responsive prostate cancer cells and thereby promote recruitment of monocytes. Given the inhibitory effect of broccoli-derived compounds indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM) on androgen-dependent pathways, we also reasoned that I3C and DIM could modulate the effect of androgen on CCL2-mediated pathways. Dihydrotestosterone was found to induce a time-dependent (0–72 hours) and concentration-dependent (0–1 nmol/L) increase in CCL2 mRNA levels in androgen-responsive human prostate cancer cells (LNCaP). This increase in CCL2 mRNA corresponded with increased secretion of CCL2 protein. The effect of dihydrotestosterone was mediated through an androgen receptor (AR)-dependent pathway as small inhibitor RNA against AR negated the induction of CCL2. Although dihydrotestosterone also induced TWIST1 mRNA, an epithelial-mesenchymal transition-related factor, and purported inducer of CCL2, blocking its expression with small inhibitor RNA did not inhibit dihydrotestosterone induction of CCL2 mRNA. Moreover, conditioned media from androgen-treated cells promoted human monocyte THP-1 cell migration and this effect was blocked by antibody against CCL2. Both I3C and DIM inhibited promotional effects of dihydrotestosterone on CCL2 and migration. These results show that androgen may regulate CCL2 and promote inflammatory micro-environment in prostate tumors and that this process can be blocked by broccoli-derived compounds. *Cancer Prev Res*; 6(6): 519–29. ©2013 AACR.

Introduction

Androgens are functionally required for the normal growth and development of the prostate gland. In adult males, androgens promote secretory epithelial cell survival. However, androgens also promote prostate tumor development and progression (1, 2). Androgen deprivation is the only clinically effective therapy for advanced prostate cancer. However, because of the relapse of castration-resistant

androgen-independent tumors, the long-term benefit of androgen deprivation in patients with metastatic disease remains controversial (3, 4). Although previous research shows that many of the biologic effects of androgen are likely through the regulation of androgen-responsive genes (ARG) via an androgen receptor (AR)-mediated pathway (5), some of the molecular effects of androgens in normal and prostate cancer remain unresolved.

Inflammation as a causal agent has been linked to approximately 20% of human cancers (6). In prostate cancer, a growing amount of evidence suggests a link between prostate inflammation and subsequent cancer development (7–12). Previous studies suggest that C-C chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1) may play pivotal role in prostate cancer tumorigenesis and invasion (13, 14). CCL2 is known to attract monocytes to the site of inflammation and, by binding to its receptor CCR2, directly stimulates prostate cancer cell proliferation, survival, and migration (15). Prostate cancer cells LNCaP, C4-2B, PC-3, and VCaP produce CCL2 (16, 17). Furthermore, recent findings suggest a role for CCL2 in acquisition of epithelial-mesenchymal transition (EMT) properties (18). EMT has been shown to be crucial for the pathogenesis of tissue fibrosis and cancer (19). Prostate tumor epithelial cells gain the ability to migrate and invade

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My view of 21st Century Nutrition Research

Systems Nutrition, 4P Nutrition

4 New Ps in Nutrition

1. Predictive Nutrition refers to the development of a probabilistic health projection for a person based on their DNA and protein expression.

2. Preventative Nutrition denotes the creation of **Dietary practice** that will prevent a **disease/excess/deficiency** that a person is assessed to have a high probability of developing.

3. Personalized Nutrition refers to treating an individual based on their unique human genetic variation, complementing the predictive and preventative efforts above

4. Participatory Nutrition denotes a patient's active, informed involvement in their medical choices and care, acting in partnership with their health providers.

“Building Partnership !”

John Milner