



Puzzle pieces show portions of a diagram of biological interactions in human aging, from cellular pathways to whole body effects. The diagram was created by John Furber, founder of Legendary Pharmaceuticals. A complete image can be found at <http://www.legendarypharma.com/chartbg.html>. Courtesy of John Furber.

Calment's extraordinary vigor illustrates why aging researchers are so interested in studying the oldest among us, from spry seniors to long-lived yeast (*Saccharomyces cerevisiae*). We all want to know what keeps them ticking.

Any number of theories have been put forth to explain aging and identify its underlying causes. Many of them sound probable. "But the theories are way, way in front of the data," says **Stuart Kim, PhD**, professor of developmental biology and genetics at Stanford University. "Understanding aging is really in its beginning stages. I feel we aren't anywhere near a good understanding."

One thing is certain: genes play a role. They may or may not direct aging, but they do influence it. In the late 1980s, researchers reported in *Genetics* that a mutant of the *age-1* gene significantly extends the lifetime of the nematode worm (*Caenorhabditis elegans*). Since then, genetic researchers have been pinpointing other single genes that either slow, speed up, or alter the aging process. The count is up to more than 100 in the worm. A few age-associated genes have even been identified in humans.

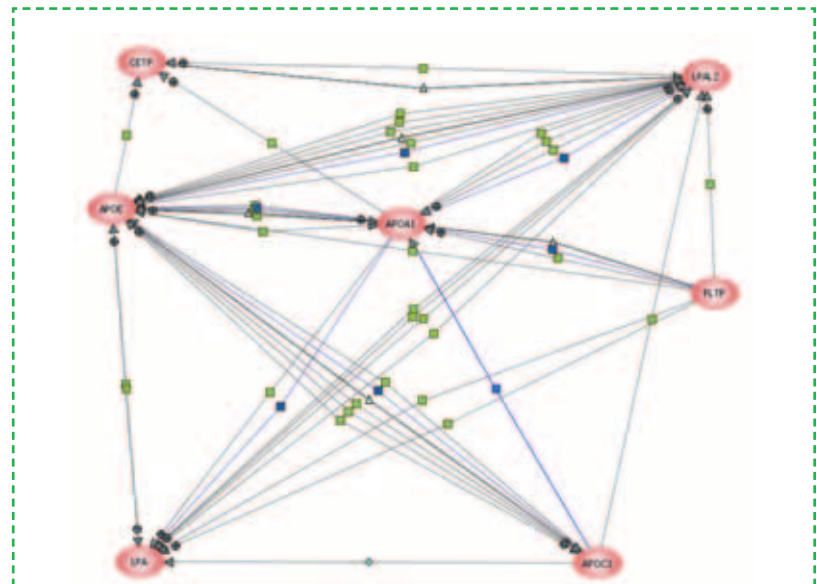
"But no one really knows how they fit together," says **Scott Pletcher, PhD**, an assistant professor in the Huffington Center on Aging at Baylor College of Medicine. The challenge now, he says, is to take that growing stack of largely disconnected single genes that affect aging and start putting them together.

Computational tools are helping aging researchers take that next step. The complexity and variability of aging itself, along with the fragmented nature of researchers' current understanding of aging, call for tools that can help scientists dig through mounds of data to find often subtle connections. Within the fields of comparative genomics, bioinformatics, and systems biology, for example, tools are now being developed and used to help assemble the puzzle of aging.

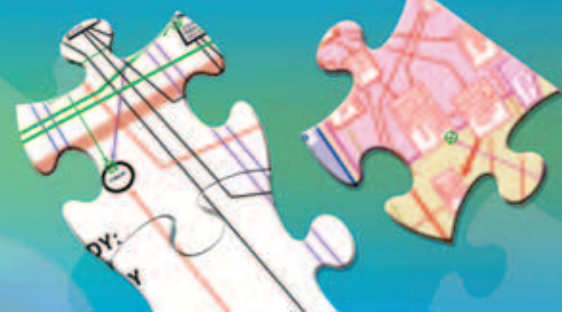
BUFFERING GENES IN CENTENARIANS

Nir Barzilai, MD, director of the Institute for Aging Research at the Albert Einstein College of Medicine, studies a group of more than 400 Ashkenazi Jewish centenarians and their children.

Barzilai and his colleagues have identified a number of longevity genotypes concentrated in this population, including a beneficial variant of CETP (the gene for cholesteryl ester transfer protein) associated with a decreased risk of heart disease. Barzilai has also noticed, however, that while centenarians are enriched with longevity genotypes, they also often concurrently harbor genotypes linked to early deaths from the diseases of old age. It seems a paradox. Barzilai and his colleagues guessed that this might occur because longevity genes buffer centenarians against the effects of the age-related disease genes.



This protein-protein interaction map indicates that the proteins lipoprotein(a) (LPA—lower left) and cholesteryl ester transfer protein (CETP—upper left) indirectly interact. This map was created from a search of the biomedical literature performed by Nir Barzilai and his colleagues after they determined that the favorable (CETP) genotype neutralizes the harmful effects of the unfavorable lipoprotein(a) genotype. The team used the GRID (General Repository of Interaction Database) to build the network and confirmed their findings using Pathway Architect software by Stratigene. Courtesy of Nir Barzilai.



Genes influence lifespan. When asked why they live to be so old, centenarians will say, "What do you mean, live to be so old? My mother was 102, and my grandfather was 108," says Nir Barzilai who studies large groups of the oldest of the old. "Well, we say, 'you have to tell us, did you eat yogurt all your life? What did you do?' ... But they didn't do anything special." Not one of them exercised regularly, a third were obese or overweight, and about a quarter of them smoked. "They have a family history, they didn't interact with the environment, and they are 100 years old. So we expect to find on the genomic level something different between them and 60-year-olds."

To test their hypothesis, they came up with a creative systems approach to help untangle the interaction between longevity genes and their counterparts. The results appeared in *PLoS Computational Biology* in August 2007. If, they said, a gene encourages longevity, it ought to be more common in older populations.

If, on the other hand, a gene makes seniors prone to disease, the individuals with the ill-fated gene will die off, and its frequency in the population should dwindle with age. But if an age-related disease gene first falls and then climbs back up in older populations—showing a U-shaped frequency curve—then a longevity gene in the population could be buffering the effects of the age-related disease gene. The longevity gene protects its holders from the disease gene's harmful effects, allowing the disease gene to remain well-represented in the long-lived crowd.

Barzilai and his coworkers gathered 1.6 million points of data on genes related to cardiovascular disease in 1200 centenarians, their children, and unrelated controls. Then the researchers looked for patterns in gene frequency.

The U-shaped frequency curve turned up for a gene called LPA, which codes for lipoprotein(a) and is associated with an increased risk of vascular disease in the elderly. The frequency of the LPA gene decreased until age 80-85, but subsequently increased until, by age 100, its prevalence was similar to that found in 60-year-olds.

Examining frequency trends in sub-populations, the researchers carefully looked for specific buffering of LPA by longevity genes. Their hypothesis was correct. They found that those with the CETP genotype for longevity can also have the detrimental LPA genotype but it will not shorten their lives.

Barzilai writes, "it is most likely that longevity involves a far more complex relationship among longevity and disease genes than the pairwise interactions we have introduced here." But his approach is a start toward unraveling the complicated connections between human aging and longevity genes, and a "proof of concept" for applying high-throughput methods to identify gene-gene interactions such as buffering.



HIGH-DIMENSIONAL BIOMARKER

The growth of data-rich gene and protein profiling tools has instilled some new hope and creativity in the search for biomarkers of aging. A substantial drag on the field of aging is that experiments take so long. A typical experiment involves altering the genes, diet or lifestyle of an animal and observing it till death. “For mammals, that’s going to be years,” Kim says. “For humans, it’s decades!”

“What we need,” he says, “is some way to find out right away whether we’ve made an animal younger or older.” In other words, the field needs a biomarker of aging.

The fact that aging researchers don’t yet have biomarkers isn’t for lack of trying. A ten-year effort spearheaded by the National Institutes of Health in 1988 to find biomarkers of aging in the mouse failed. Many potential measuring sticks have been put forth for both animals and humans, including hormones, body temperature, telomere lengths, and immune cell concentration in the blood. Yet these single candidates generally display too much variation in a healthy, non-aging population, let alone an aging one.

Kim believes he is now onto something with his recent work on the transcriptional profile: a snapshot of the genes being expressed in a group of cells at any one time, usually obtained with microarray technology.

Since a transcriptional profile is a “fairly complex readout from hundreds and hundreds of different markers,” Kim says, it provides far more information than a single biomarker could. “It’s by integrating all of those different markers together that we have greater power to predict physiological age,” he says.

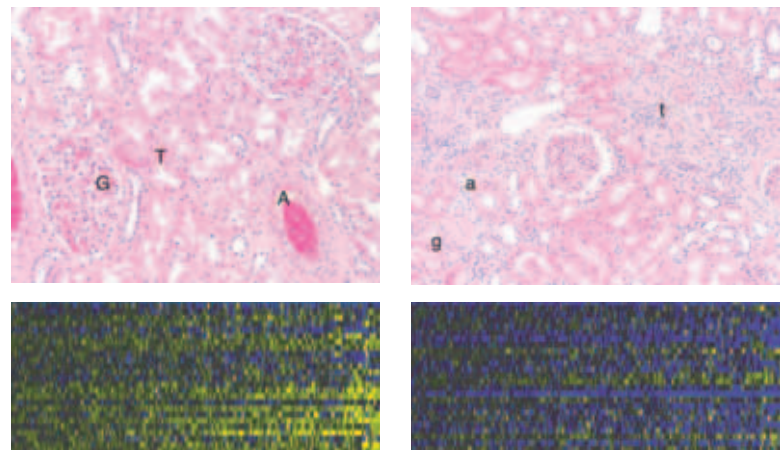
Kim started by looking at old and young human kidneys. Like other filters, the key components of a kidney start to clog as we age: the main filtration unit, the arteries, and the excretion tubules. But some kidneys age much faster than others. Indeed, a physician can simply look at a kidney tissue sample and tell its physiological age—a measure that can be out of sync with chronological age and serves as a more accurate measure of kidney health.

The Stanford group gathered transcriptional profiles from 74 kidneys and found, in a study

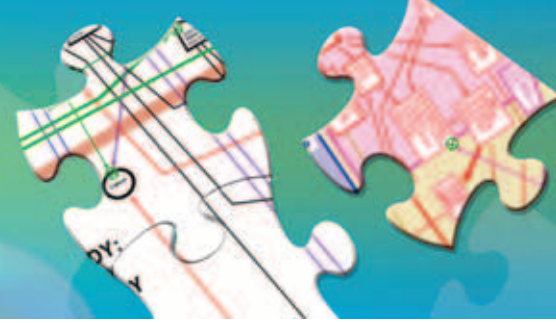
reported in December 2004 in *PLoS Biology*, that 447 genes (out of 33,000 tested) showed a difference in regulation with chronological age. And when Kim placed the doctor’s measures of physiological age against the age indicated by the transcriptional profiles, they correlated. One 78-year-old woman’s kidney that looked like it belonged to a 100-year-old displayed a very old transcriptional profile. And an 81-year-old man’s kidney that looked “terrific” showed a much younger transcriptional profile.

Kim repeated the transcriptional profile study in human skeletal muscle. Again, the results (published in *PLOS Genetics* in July 2006) showed that the transcriptional profiles reflected real physiological age (muscle deterioration), “not just the birthdate.”

Identifying an aging biomarker for a specific tissue like kidney or muscle is helpful. Even more helpful would be a biomarker for a whole organism, especially one that crosses species. In search of a more universal biomarker, Kim also performed a meta-analysis of aging transcriptional profiles in four species. He set his human kidney and muscle data against fly, worm, and mouse data from his and other laboratories.



Kidney age can be tracked by tissue inspection or gene expression profiles. A 29-year-old kidney (top left) with a normal filtering unit (glomerulus, G), tubules (T), and arterioles (A) shows a young gene-expression profile (bottom left). An 84-year-old kidney (top right) with a hardened glomerulus (g), narrow tubules (t), and diseased arteries (a) displays an old gene expression profile (bottom right). The genes shown drop in expression with age. Yellow indicates high expression; blue indicates low expression. Courtesy of Stuart Kim.



To measure aging in individuals, Mitnitski and Rockwood created a frailty index based on the number of aging indicators possessed by each individual. A sample collection of 20 indicators, also called deficits, was published in *BMC Geriatrics* in February 2002. It includes vision loss, hearing loss, impaired mobility, vascular problem, gait abnormality, impaired vibration sense, difficulty toileting, difficulty cooking, difficulty bathing, difficulty going out, difficulty grooming, skin problems, resting tremor, changes in sleep, difficulty dressing, urinary complaints, gastro-intestinal problem, diabetes, hypertension, and limb tone abnormality. Each indicator is given equal weight in the index, whether it be diabetes or a skin problem.

For this analysis, Kim and his coworkers designed a set of statistical methods to help overcome a “pervasive methodological challenge in genomics studies”—that the simultaneous reading of tens of thousands of genes increases the possibility of a random, rare event showing up as statistically significant. They categorized 20,000 genes by pathways into 600 gene sets, and then looked for overall trends within the gene sets. Their analysis identified only one pathway that showed the same trend in all four species: Expression of genes in the electron transport chain decreased two-fold over the lifespan of each animal.

Why the electron transport pathway would be the only shared aging pathway across four species is still unknown, Kim says. “But it looks like an unavoidable consequence of getting old.”

Other laboratories have completed similar cross-species comparisons, and still others are gathering data for such a comparison. Results have been mixed so far, but if future studies confirm Kim’s results, a profile of gene expression in the electron transport chain may be a candidate for a universal biomarker of aging.

QUANTIFYING AGING

In humans, chronological age is often used as a rough measure of the aging process. A young actuary in the early 1800s, studying mortality data in England, discovered that risk of death in populations rises exponentially after puberty, doubling about every seven years. Benjamin Gompertz’ curve, called the Law of Mortality, links chronological age to risk of death, and has proven useful to insurance companies and aging researchers ever since.

Yet, says **Arnold Mitnitski, PhD**, “individuals do not die from old age. They die because they become more vulnerable, and this occurs with age.” Mitnitski, an associate professor of medicine at Dalhousie University in Canada, and his colleague **Kenneth Rockwood, MD**, a professor of geriatric medicine and neurology, developed a better measure of aging for individuals than chronological age—a measure that takes individual health vulnerability into account. They call it the frailty index.

When they first attempted to quantify biological age, the researchers had access to a large

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Canadian database tracking the medical status of folks as they aged. Mitnitski asked which signs or symptoms listed in the database are essential to tracking aging. Is it incidence of cancer? Is it declining mobility? The researchers tried to use a classical statistical approach to determine which variables were most significant. But the approach assumed that the variables were independent. And Mitnitski and Rockwood’s analysis, to their surprise, revealed that every indication of health from declining eyesight to memory loss was just as important as every other one in tracking aging.

Aging is like other complex systems, the scientists realized, in which all variables are so inter-related, they are too difficult to treat separately.

But Mitnitski and Rockwood still wanted to somehow put the variables together into a quantifiable indicator. So they simply counted the characteristics up. They listed every possible sign or symptom of aging, called them deficits, and added them up for each individual.

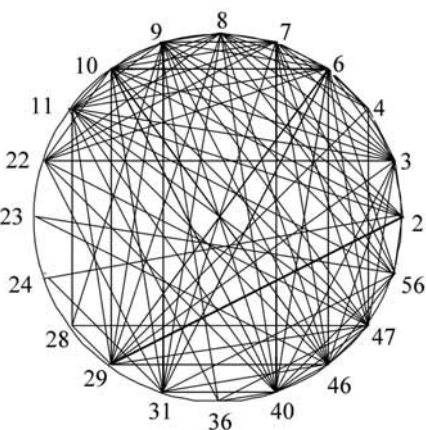
“Usually nobody does that, just counts,” Mitnitski says. But it worked. The number increased with age. And it increased exponentially. It looked, to Mitnitski’s surprise, like Gompertz’ Law of Mortality.

“It was bizarre that it worked,” Mitnitski says. It seemed crazy to give a characteristic like cancer the

same weight as a skin condition or a change in sleep patterns. But they found that weighting the deficits was unnecessary. It seems that each of the variables is so highly connected to all the others that it doesn’t matter which variables are picked; they all indicate the underlying process of aging.

In order to use the measure across different datasets, the scientists changed their simple sum of deficits into an index measure: a ratio between zero and one. Then they tested the index on datasets from Sweden to Australia. They found it doesn’t matter which aging indicators are used—and the more, the better.

Mitnitski and Rockwood’s frailty index might have important theoretical implications. Now that Mitnitski and Rockwood have a way of expressing health status as a single number, they are analyzing individual health trajectories over time. Health status tends to go up and down with an overall downward trend, so they have found it can be modeled as a stochastic (random) process. “This makes it possible to apply a powerful mathematical apparatus developed in other fields such as systems theory and engineering to the problems of aging,” Mitnitski says. “Our approach opens new horizons in the application of mathematical modeling to aging and health issues.”



The signs and symptoms of aging are highly interconnected. Each node number on the edge of the graph above is one of the deficits listed in the previous image; lines represent statistically significant relationships between deficits. Courtesy of Arnold Mitnitski and BMC Geriatrics.

“We start with simple mechanistic models, and then join models together to examine interacting effects,” says Carole Proctor.



AGING IN SILICO

The complex nature of aging has also been tackled using computational simulation. **Thomas Kirkwood, PhD**, director of the Institute for Ageing and Health at Newcastle University, started making computational models of molecular aging processes more than fifteen years ago.

Kirkwood is motivated in part by a desire to simulate some of the heaps of theories about the mechanisms behind human aging. Some researchers say that hormones are behind aging, others that accumulated damage to DNA leaves the body increasingly vulnerable. Aging could result from growing oxidative damage, an increasing imbalance between launching inflammatory reactions and fighting inflammation, or a shortening of the telomeres (protective caps at the tips of chromosomes). Perhaps aging is a gradual loss of transcriptional control, or a build-up of random damage to the short-lived, low-affinity interactions crucial to operational cellular networks. Perhaps an older body must compromise in its ability to repair itself. Perhaps it is a bit of all of these.

Kirkwood's group has modeled aspects of several of these. For example, they looked at how telomere shortening might be influenced by oxidative stress. They modeled how malfunctioning mitochondria, aberrant proteins, free radicals, and scavengers might interact in the aging cell. And, more recently, they simulated the role of molecular chaperones in aging, and how the ubiquitin-proteasome system impacts protein degradation in aging.

From the start, Kirkwood and his group have created simulations like modular furniture: pieces that can easily be modified, put together, and taken apart again to test the interconnecting pieces underlying aging's complexity. "We start with simple mechanistic models, and then join models together to examine interacting effects," says **Carole Proctor, PhD**, a research associate in the group.

The Newcastle group has also created a model-building platform that allows other researchers, even those not so familiar with modeling, to simulate aging processes. The eventual goal of the Biology of Ageing e-Science Integration and Simulation System (BASIS) project is to put together a simulation of a virtual aging cell,

Less-Fuss Meta-Analysis Building the Gene Aging Nexus

Comparing microarray data in the field of aging (and other fields for that matter) can be a nuisance. Different chips or cDNA arrays generate gene expression data in different ways. Even if the array is the same, different parameters set by different laboratories can create widespread variation that is difficult to account for.

Caleb Finch, PhD, professor of gerontology and biological science at the University of Southern California, frustrated when he was unable to even "connect successive papers from the same laboratory," decided to construct a data-mining platform that could assemble databases of primary reads of the chips, convert them into a common format, and offer a variety of statistical tools for cross-examination of the experiments.

He collaborated with a fellow researcher at USC, **Xianghong Jasmine Zhou, PhD**, an assistant professor in molecular and computational biology, and the result is the Gene Aging Nexus (<http://gan.usc.edu/public/index.jsp>), or GAN. GAN currently holds data from more than 800 microarray experiments in six species (human, rat, mouse, fly, worm, and yeast). And it offers tools to visualize the data with charts and graphs.

When Gene Aging Nexus was introduced in *Nucleic Acids Research* in November 2006, **Chris Patil, PhD**, a postdoctoral fellow studying the genetics of aging with **Judith Campisi, PhD**, at Lawrence Berkeley National Laboratory, gave it a hearty welcome on his aging blog (<http://ouroboros.wordpress.com/>). "As anyone who's tried to compare experiments performed on different arrays in different cell types (or species) in different labs can attest," Patil wrote, "this publicly available resource will be a huge boon to meta-analysis of gene expression data related to aging."



Although scientists have pinpointed only a handful of human genes that directly affect human aging, one group created a database of more than 200 genes possibly involved in human aging. GenAge is a collection of either human homologs of animal genes known to be related to aging, genes that are part of networks known to be associated with the process of aging, or genes of other interacting partners of age-associated proteins. **João Pedro de Magalhães, PhD**, a postdoctoral fellow at Harvard University who maintains the database, also helped create a protein interaction network (left) of all the proteins from GenAge known to interact. The colors represent functional groups: red, DNA repair, replication, and condensation; blue, growth and development; yellow, transcriptional regulation and signaling; orange, redox and oxidative regulation and stress response; green, apoptosis; grey, unknown or other. GenAge is available at <http://genomics.senescence.info/genes/>. Reprinted from "GenAge: a genomic and proteomic network map of human ageing," *FEBS Letters* 571(1-3) 2004, with permission from Elsevier and co-author João Pedro de Magalhães.

virtual aging tissues (starting with fibroblast connective tissue, intestinal epithelium, and neuronal networks), and virtual aging organisms (starting with *C. elegans*).

Their goal of simulating aging at the cell, tissue and organism level reflects a growing desire within the field of aging to expand from a largely molecular focus to a systems-level exploration.

AGING SYSTEMS

Alterations that begin on a molecular level eventually cause the skin to droop, the kidney's filtering to falter, fat cells to redistribute, and bones to become more porous. "Changes percolate upward with age," says **Andres Kriete, PhD**, associate professor for bioinformation engineering at Drexel University.

Kriete believes that quantifying the touches of aging on multiple levels, from the cells to the whole organism, and then integrating that evidence using models and other forms of computational analysis, will be crucial future steps for understanding aging.

"We need a comprehensive point of view," says **Claudio Franceschi, PhD**, professor of immunology at the University of Bologna. "We should continue to do molecular biology studies, because unless you have the molecular biology and genetics studies that identify pathway molecules, then you are lost. But you also need to understand what is going on at more integrated levels. You really need systems and computational biology. Otherwise, you see the details but lose the entire picture."

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Large-Scale Data Mining of Centenarians

For many years, says Franceschi, centenarian researchers studied the genetics of longevity in groups of centenarians about 200 or 300 strong. "But we realized this was not enough," he says. Much larger studies were needed to account for the huge variability among centenarians and gain the statistical power for significant results. Old age is very noisy; many combinations of genetics, lifestyles, cultural factors, and randomness can lead to longevity, Franceschi says.

So Franceschi worked to set up a large-scale

One unique and powerful aspect of the GEHA analysis is that demographers in the consortium will weight the relative significance of each sibling pair's genetic information according to demographic data. For example, the genetic information of two centenarian brothers in Poland will be given slightly more weight than the genetic information of two centenarian sisters in Italy. Men reach 100 less often than women and Poles reach 100 less often than Italians. Because of that, the Polish brothers' genetic data should tell the researchers more about longevity than the

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project that would eventually include 24 research groups in Europe and one in China working together to sort through the genomes of 5,300 people in 11 European countries. Half of the subjects of the study are siblings over 90 years old. (A few recruited families have five siblings over 90!) GEHA (GEnetics of Healthy Aging) is the largest initiative on the genetics of human longevity ever.

To search for candidate longevity genes, the consortium will perform a linkage analysis of each sibling pair's genomes using 25,000 DNA markers per genome. A linkage analysis calculates which markers are shared more than by chance. "Since they share a trait, which is longevity, we assume that they will share more than 50% of those polymorphisms or markers in their genome that are related exactly to longevity," Franceschi says.

Italian sisters' data. "The genetic data will be corrected in a way," Franceschi says, taking into account the odds that each sibling pair had to beat to reach their current ages.

GEHA collaborators will also stratify the data according to health status and cognitive and functional abilities. Some reach 90 in good shape, and some are in very poor shape. "One is associated with healthy aging and the other is associated with aging with some problems," Franceschi says. "We want to account for that."

Franceschi likens GEHA to the large coordinated research efforts of physicists at CERN or the Stanford Linear Accelerator Center. Aging needs such an effort, he says, because lack of statistical power is the most common problem in studies of complex genetic traits such as human longevity. The first results from GEHA should arrive in spring 2008.