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Big gene banks: nuggets for drug discovery or fool's gold?

J.C. Louis, freelance writer

Do gene-mapping studies that look for complex traits among human populations hold promise for identifying drug targets? The best known of these studies, undertaken in Finland and Iceland, have certainly deepened our scientific understanding of the causes of disease. However, some experts outside the industry are now questioning whether the payoff in treatment will ultimately justify the expense.

'Everyone in the world is trying to find genes and appropriate them to complex diseases, and then do epidemiology on cohorts to find differences between biological and environmental components,' says Claude Laberge, Director of the Quebec Network of Applied Genetic Medicine (http://www.rmga.qc.ca/default.htm), which recently issued its *Principles on Human Genetic Research Involving Populations*. 'This is good for finding genes,' he says, 'but afterwards someone must put the findings to use in the general population. He continues, 'Just where will this new knowledge be important? In prevention, promotion of treatment, organizing access to genetic services, or to customize or personalize a type of treatment?' The answer is unclear.

Benefits of large-scale studies

Large-scale population studies are numerous, from those involving the Amish and Mennonites, to the Pima Indians of Arizona, the Sardinians and the Bedouins. Those conducted in Finland and Iceland are perhaps the most substantial examples. With stable cultures and demographic registries, they approximate the usefulness of inbred animal strains to genetic research. For example, Leena Peltonen a human geneticist of the University of California, Los Angeles (http://www.ucla.edu), and the University of Helsinki (http://www.helsinki.fi/english), notes that these populations have a high prevalence for certain diseases, more inbreeding (the best conditions for mapping recessive genes), a more uniform genetic background and good genealogical records.

Certain hallmarks of Finland's demographic history – few original founders, subsequent isolation, rapid expansion and bottlenecks that contribute to genetic drift – have helped to create a unique catalogue of genetic diseases. Finnish records include a large proportion of the total population of 5.1 million.

Iceland, founded by a small number of ninth-century settlers from Norway, Ireland and Scotland, has experienced little immigration over the past 11 centuries. Its 1000-year file of genealogical records provides unique sources for tracking hereditary diseases.

Mapping gene location

The private Reykjavik company, deCode Genetics (http://www.decodegenetics.com/), makes the most of these historical records. The Icelandic government has licensed deCode to build and operate a database based on a detailed genetic study of the population.

In recent months, deCode scientists have announced their mapping of the locations of errant genes for numerous

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common multigenic diseases, including Alzheimer's disease, asthma, hypertension and schizophrenia. deCode's linkage-map study has drawn praise from a leading researcher who worked on the Finnish study.

'If anyone has a chance to map things, it is them!' declares Joseph Terwilliger, Associate Professor of Genetics at Columbia University (http://www.columbia.edu). 'Use of those genealogies, as we did in Finland, is a wise, well-thought-out strategy.'

Interpreting the results

The studies are both notable in design and compelling in results, says Nancy Cox, Professor of Genetics at the University of Chicago (http://www.uchicago.edu). However, she adds that attributing the results to a millennium's worth of genealogical data might be an exaggeration that would deter commercial competitors.

'Do they need 11 to 12 generations?' she asks. 'People would like to replicate their success; the genealogies cannot be replicated. If three or four generations is all that's necessary, then a lot more places could do similar studies.'

The best way to know the potential of these results is if competitors believe in it, Terwilliger says, 'and to the best of my knowledge, their competitors are not exactly overwhelmed by the evidence to date.' He also 'strongly questions' some of the statistical analysis. As to the 'schizophrenia discovery', he notes, the company claims to have found 'a variant with extremely small effect on risk of schizophrenia'. In Finland, says Terwilliger, the sibling risk of schizophrenia is only 4%, so most of the cause of schizophrenia must be environmental. So, what is the use of the gene?

Some geneticists argue that the gene could be used to learn about mechanisms and, subsequently, to help identify pathways that could lead to useful drug targets. However, Penn State University (http://www.psu.edu) anthropologist and geneticist Ken Weiss argues, 'we do not have to learn of pathways by conducting small founder, big family or population studies. We have many ways to find variants in genes that are causal for complex diseases or to identify a pathway'.

Common complex diseases

Weiss believes that the likelihood of finding clinical benefits justifies such studies in small, relatively homogenous founder populations in the clear-cut case of rare diseases. However, for common, complex diseases – the very ones for which deCode is proclaims has big potential – the issue is whether the sample population presents (and the study can identify) 'variants that are common enough to be biomedically useful'.

In other words, is the population of Iceland homogeneous enough to provide useful clues for discovering the genetic roots of common diseases in more heterogeneous societies? Cox believes that if there is a lot of genetic heterogeneity, where different individuals derive risk from different individual genes, perhaps only 10% of the genes would present druggable targets. 'That would be less optimistic than if a complex phenotype arises as a consequence of the interaction of many genes, where a single particular gene presents an adequate target,' she says. 'That situation might enable one to more effectively intervene and lower the overall risk.'

Terwilliger and Weiss, however, remain more skeptical. 'Neither Finland nor Iceland is so isolated as to make a difference,' Terwilliger maintains. 'There are a lot of exposures, lots of variety in food and diet, and lots of other risk factors.' If causation is complex, or if the variations they find are unique to Iceland, Weiss predicts, the studies will not be broadly applicable.

Unnecessary investment?

The time-honored premise behind genetics research is that it will someday lead to 'magic treatments' by yielding a continuous succession of candidate genes for complex disease. However, Weiss argues that the 'self-perpetuating cycle of huge investment' in huge genetic databanks will not ultimately deliver what is promised: a treatment for each major disease, based on genetic research.

'We have very few successes, even with single gene diseases,' Weiss declares. 'Why chase tens or hundreds of candidate genes, when we do not know what to do with the ones we have?'

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Cleaning up the environment

Paul D. Thacker, freelance writer

One vexing problem with cancer is that even if the disease appears to be in remission it often comes back. Although it has long been suspected that cancerous cells were modifying their environment, this was not well understood until 2001 [1]. Mary Hendrix, Department Chair of Anatomy and Cell Biology at the University of Iowa (http://www.uiowa.edu), discovered that aggressive melanoma cells overexpress certain proteins, which modify surrounding cells. Her group has followed up this paper with another, which shows that COL-3, a chemically modified tetracycline (CMT), inhibits both expression of these proteins and their action on surrounding cells.

Creating a favourable habitat for cancer

Aggressive melanoma cells overexpress several proteins, including lamin 5 (Ln5) γ2 chain, which is overexpressed 50-fold in aggressive cells but not produced by non-aggressive cells. The aggressive cells also overexpress metalloproteinases (MMPs), such as MMP2 and membrane type 1 (MT1)-MMP. The proteins then act co-operatively to change the tumour environment by cleaving the γ-chain. The fragments are then incorporated into the extracellular matrix.

The evidence shows that these fragments can induce aggressive behavior in surrounding cells, causing them to engage in vasculogenic mimicry when cultured on a 3D matrix [1]. Vasculogenic mimicry refers to the ability to express endothelial and vascular associated genes including many MMPs and vascular endothelial (VE)-cadherin. The Hendrix group discovered this effect by studying non-aggressive MUM-2C melanoma cells, which were grown on a 3D collagen matrix that had first been used by aggressive MUM-2B cells. The 3D matrix helps to replicate an environment that is more similar to an organ than a petri dish, thus enabling the cells to grow and interact more naturally.

'We put in the aggressive cells first and they leave behind a microenvironment,' explains Hendrix. 'Then we remove the aggressive cells and put in the non-aggressive cells and you see induction.'

Balakrishna L. Lokeshwar, Associate Professor of Urology at the University of Miami (http://www.miami.edu), explains that the microenvironment probably ensures that removing the tumour only causes other cells to grow in its place. 'This concept of the tumour microenvironment is similar to a plant making it's own fertilizer,' he says. 'Once you remove the plant, you leave behind the fertilizer and the other surrounding plants now start growing faster.'

Targeting the microenvironment

To inhibit microenvironment signaling, Hendrix chose COL-3, a chemically modified tetracycline that has antimetastatic [2,3] and antiangiogenic activity, and also inhibits MMP activity [4].

In the experiment, collagen plates were conditioned by aggressive MUM-2B cells, which were removed after four days. During this time, the cells overexpress Ln5 γ-2, which is cleaved by the MMPs. The γ-chain fragments then incorporate themselves into the collagen. Following removal of the MUM-2B cells, non-aggressive MUM-2C cells are seeded onto the plate and begin to grow in a network pattern that indicates an aggressive phenotype. The MUM-2C – which does not normally produce Ln5 γ-2 chains – also began producing the protein.

However, when MUM-2B cells were grown with COL-3, the cells changed and no longer grew in a network pattern. With PCR analysis, it was also found that they were inhibited from producing many vasculogenic markers, including MMP2, MMP9 and cadherin. Further, when the collagen matrix was tested, it was found to no longer include γ-2 signaling fragments, and when seeded with MUM-2C, the non-aggressive cells did not grow in a network pattern, nor did they produce Ln5 γ-2.

'The study shows that not only does COL-3 inhibit expression of vasculogenic markers, but it also inhibits cleavage of Ln5 γ-2,' says Hendrix. 'That means there is an inhibition of these signals which aggressive cells leave behind in the environment.'

Implications for future therapies

Describing the study as 'extremely elegant,' Lokeshwar says microenvironment work holds the possibility of combining new drugs with standard chemotherapy to wipe out these signals. 'The microenvironment is a hot new field because we've seen the failure of standard chemotherapy, especially with high density tumours.'

What is not well understood is the mechanism for COL-3 activity. As the Hendrix paper shows, the drug inhibits protein synthesis, but it's ability to block MMP enzymatic action is thought to be caused by chelating divalent cations. Both MMPs and COL-3 chelate these cations, with COL-3 being the better binder.

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