Systems approaches for pharmacogenetics and pharmacogenomics

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'Rather than linking a phenotype to a single gene, the new paradigm may be linking a trait to a pathway or a biological system.'

The search for genetic variants that influence medically-relevant phenotypes has been driven by the central dogma of genetics: DNA encodes genes as discrete units of inheritance; genes encoded by DNA are transcribed to RNA; messenger RNA molecules are translated to proteins; one protein has one function; and aberrant proteins encoded by mutant gene variants lead to aberrant biological activity. This paradigm explains many human diseases [1] and has been extended to diseases where two or perhaps three genes contribute [2].

Unfortunately, the paradigm of 'one gene, one protein, one function' has failed to reveal the causes of prevalent complex diseases known conclusively from twin studies to have a genetic basis [3,4]. These diseases include arthritis, diabetes and risk factors for cardiovascular disease. Similarly, this paradigm may also fail for traits related to pharmacokinetics and pharmacodynamics.

Technological advances in genetic analysis now permit whole-genome association, in which the entire genome of an individual is screened for genetic variation at a series of preselected locations, termed markers. In these studies, there are approximately as many markers as individual genes, if not more. SNPs are the default choice for markers, and SNP sets for the human genome now have 100,000–500,000 markers to provide coverage of our 24,000 genes. Testing these markers for association with a phenotype is a challenging signal-to-noise problem: it is difficult to distinguish between the few real causative effects and the statistical flukes that are expected from examining 100,000 markers, whether markers are individual SNPs or correlated local patterns of SNPs termed haplotypes [5].

It is now possible to test hypotheses that phenotypes result from cumulative, subtle regulatory changes, in contrast to the catastrophic

failure of a single protein as in classic Mendelian genetics (Figure 1). Rather than linking a phenotype to a single gene, the new paradigm may be linking a trait to a pathway or a biological system.

Although linking genetics to pathways is an intriguing idea, the lack of systematic pathway maps beyond basic metabolism has presented an insurmountable obstacle. Consequently, most work in human genetics turned to other avenues; the theoretical framework for pathway-level mapping is an important current avenue of research. Only recently, in part owing to the author's work [6], have pathway maps become available to the extent that they can be used in genetic analysis.

Overview of the challenges

A schematic illustrates the challenges in understanding genetics data for a complex phenotype such as response to a drug (Figure 2). In this picture, the quantitative level of response corresponds to the quantitative level of activity of a biological network. Genetics can identify which pathway is involved, but requires several steps of inference, each with its own challenges:

- From a disease to a genetic marker (whole-genome association): identify a physical region of the genome that houses a genetic variant that affects the trait value. Challenges: regions with small but real effects (expected for complex traits) are easy to miss. Variants that interact synergistically are even more difficult to detect.
- From a genetic marker to a gene (candidate gene identification): identify the gene or genetic variant within the region that is responsible for the effect. Challenges: regions may contain several genes, making it difficult to conclusively point to the gene responsible for an effect.
- From candidate gene to system (systems biology): understand the systems-level architecture of a disease by mapping causative genes back to biological pathways. Challenges: this step lays inference upon inference because mapping markers to genes is already difficult.

Phenotype to marker: whole-genome association

- Genetic association tests are designed to identify correlations between genetic variations and phenotypes, which may either be discrete classes such as case versus control, or quantitative measures such as parameters in a pharmacokinetics/pharmacodynamics model. A marker that is associated with a phenotype might not cause the phenotype of interest. Instead, the marker might be located physically close to the functional variant so that the marker allele is tightly correlated with the

functional allele (Figure 3). The precise meaning of ‘physically close’ depends on the type of study. In family studies, correlations depend on the typical distance between meiotic recombination sites, roughly 50–100 Mb. In a population of unrelated humans, allelic correlations decay much faster, with a length scale of 10–100 kb. This linked region for a quantitative trait is termed a quantitative trait locus (QTL). Since the human genome has approximately 3 billion nucleotides, the number of markers required is of the order of 10,000–500,000.

Figure 1. Functional effects of DNA variants in protein-coding and regulatory regions.

(A) The central dogma of genetics is that DNA encodes mRNA, mRNA encodes protein, and proteins have discrete functions. Genetic diseases arise when mutant DNA encodes a protein with aberrant function (B). Disease may also arise when proteins in a pathway are not properly regulated, even though the proteins themselves may be perfectly normal (C). These changes can be due to variation in regulatory DNA that does not encode protein. Organizing genetic analysis using systems-level knowledge will identify disease-causing variants.

Figure 2. Identifying the system of genes responsible for a phenotype requires backwards inference from phenotypes to markers, from markers to genes and from genes to pathways.

When conducting a study with upwards of 1 million markers, it is important to reject false positives owing to statistical flukes that are expected to occur at roughly the one-in-a-million level, or a p-value of 10^{-6} or smaller. Establishing a real finding at this significance level for a complex trait can require studies with thousands of individuals (Figure 3). Costs for enrolling each individual in a study and performing genotyping are of the order of US\$1000 or more, for a final cost of millions of dollars. Projects like the HapMap aim to optimize the markers used [7], but do not deviate from these basic economics.

At costs of US\$1 million a study, it is essential to understand how different methods perform before applying them to human populations. Realistic assessments require the use of model organisms. Yeast (*Saccharomyces cerevisiae*) is perhaps the single most widely used genetic model organism: it is the simplest eukaryote that is widely studied; it was the first eukaryote to have its genome sequenced; and it has a panoply of genetic tools. These tools include the ability to knock out or perturb gene function [8], which is important for verifying results of genetics studies; widely available transcript profiling techniques to measure the activity of every gene at the mRNA level; and the most advanced proteomics methods to map the composition of protein complexes and pathways [9,10].

Yeast alleles and phenotypes vary between strains. By mating yeast cells from different strains, collecting the progeny, scoring the daughter cells for a phenotype and then genotyping the same cells, it is possible to identify genetic regions or loci that are associated with the same phenotype. In a well-controlled

experiment, this association establishes a causal linkage between a specific DNA region and a trait. When traits are themselves the output of a high-throughput experiment, this strategy is termed 'genetical genomics' [11].

These types of experiments have now been performed for a range of yeast phenotypes related to human disease: drug response [12], telomere maintenance relevant to cancer and aging [13]; and aberrant cellular shape or size [14].

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An influential series of experiments used gene regulation as a trait, with roughly 6000 phenotypes measured in parallel, each phenotype corresponding to the mRNA abundance of each yeast gene [15,16]. The DNA region causally linked to an mRNA is termed an expression quantitative trait locus (eQTL). Since variation in gene regulation contributes to complex disorders (Figure 1), mapping eQTLs is very relevant to human disease [17]. Mapping eQTLs in model organisms has identified DNA regions relevant to obesity [18] and other disorders [19].

A method successfully used to improve statistical association has been dimensional reduction. Traits that are highly correlated, for example, genes whose expression levels track each other closely, can be tested as a batch. This reduces the multiple-testing correction. Standard statistical techniques, such as principal components analysis [18,20] and clustering [17,21,22], have been used to reduce the number of tests.

Figure 3. The mathematics of association tests.

An association test for a complex quantitative trait can be performed as a regression test of trait value against allele dose (number of copies of a particular allele for a specific marker). The fraction of variance explained by the gene is denoted as R^2 , corresponding to a regression line with correlation R . A simple equation relates the effect size, the number (n) of individuals genotyped, and the expected statistical significance: $z^2 = NR^2$, where z is the number of standard deviations away from the null expectation for a 50:50 chance of detection. A p -value of 10^{-6} corresponds to 4.8 standard deviations, and R^2 for a complex trait can be 1% or lower. For this typical example, 2300 individuals are required just to have a 50% chance of a significant finding.

Spurious associations may arise from the technologies used to measure transcript levels. The most common method used to measure transcript levels is hybridization to wild-type sequences on a chip. If a DNA variant hybridizes less efficiently, that gene will appear to be under-expressed, even when it is present at normal levels. These variants can be correlated with nearby variants used for markers, leading to a false-positive association. This problem has been recognized [23,24]. Allele-specific expression can be used to distinguish between real *cis*-regulatory variation and a spurious effect [25,26].

Most association studies focus on main effects of a single variant, equivalent to a statistical test for correlation between trait value (gene-expression level for an eQTL) and genotype at a single marker. A standard statistical technique to identify additional markers is to subtract the effect of a marker, then identify other markers that may have smaller effects. This is equivalent to a forward regression model based on partial correlation coefficients. An additional complication is caused by epistasis, in which the effects of multiple

variants combine nonlinearly. This is equivalent to adding interaction terms to a multiple regression model. Multimarker tests have been difficult to apply in practice because, with 6000 markers as in the yeast studies, each additional marker requires an additional 6000-fold correction to a p -value. Human studies, with 100,000 markers or more, would have more difficulty to find statistical significance for any multimarker test. Calculations must also be organized for efficiency. Thus, only a few multimarker tests have been described, and to date only for yeast [27,28]. These studies have shown that multimarker effects are important, with approximately two-thirds of markers missed based on main-effect analysis alone. Furthermore, approximately half of the two-marker models have shown significant nonlinear interaction terms. Improving multimarker tests beyond brute-force methods will be necessary to identify multigene effects in humans.

Marker to gene: candidate gene identification

Identifying a physical segment of DNA linked to a trait is only the start of identifying the causative variant. Indeed, almost all of the studies cited above stop at the level of marker association, without ever identifying the genetic variant or even the gene responsible. Identifying and validating candidate genes is an outstanding challenge [29]. For eQTLs, two types of linkage are generally described, as described below.

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cis-eQTLs are markers that are physically close to the gene being regulated. The causative variant could be nucleotide substitution or insertion/deletion in the promoter region of the regulated gene that affects transcription factor or polymerase binding. *cis*-eQTLs may also arise from changes in introns or untranslated regions (UTRs) in the RNA transcript that flank the protein-coding sequence that affect RNA processing or stability. In most cases the marker is not the causative polymorphism, which remains unknown.

trans-eQTLs are markers that are far from the regulated gene. Suppose that gene *A* regulates the expression level of gene *B*. A mutant copy of

A that produces a protein with aberrant function could result in misregulation of gene *B*. A more subtle effect arises if the sequence of protein *A* is correct, but a variant upstream of gene *A* causes a change in its expression level that in turn affects the regulation of gene *B*. In this case, a *cis*-eQTL near gene *A* is also observed to be a *trans*-eQTL for gene *B*. It is difficult to go from a marker to a gene, however, because a *trans*-eQTL can be linked to several adjacent genes, with no statistical evidence to support one candidate over the others.

An intuitive explanation for a *trans*-eQTL is that it encodes a transcription factor, the class of protein most directly related to gene regulation. Unfortunately, investigations of eQTL regions have yet to show any enrichment for transcription factors. Nonsynonymous changes to protein-coding regions are another class of probable causative variants [30], but probably represent a minority of *trans*-eQTLs.

The method that has so far had the greatest success for *trans*-eQTLs is to combine genetic association with gene-expression correlation: look for a candidate gene that is close to an eQTL for a particular transcript and whose expression level correlates with that same gene [31–34]. This type of integrated analysis is promising.

Gene to pathways: systems biology

Systems biology is an integrative science that attempts to understand gene function in the context of larger biological units: protein complexes, regulatory and metabolic cellular pathway and even higher-order systems. Systems biology suggests that if one gene is implicated in causing a disease or affecting a trait, other genes in the same pathway are also likely to be involved. There is evidence that this type of reasoning can improve pathway inference using combined genetic and gene-expression data [35]. This may be an important new area for research uncovering the genetic basis for personalized medicine. Research is now needed to develop algorithms that integrate whole-genome association studies with growing knowledge of networks and pathways.

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Bibliography

- Botstein D, Risch N: Discovering genotypes underlying human phenotypes: Past successes for mendelian disease, future approaches for complex disease. *Nat. Genet.* 33(Suppl.), 228–237 (2003).
- Badano JL, Katsanis N: Beyond Mendel: an evolving view of human genetic disease transmission. *Nat. Rev. Genet.* 3(10), 779–789 (2002).
- MacGregor AJ, Snieder H, Schork NJ, Spector TD: Twins. Novel uses to study complex traits and genetic diseases. *Trends Genet.* 16(3), 131–134 (2000).
- Boomsma D, Busjahn A, Peltonen L: Classical twin studies and beyond. *Nat. Rev. Genet.* 3(11), 872–882 (2002).
- Bader JS: The relative power of SNPs and haplotype as genetic markers for association tests. *Pharmacogenomics* 2(1), 11–24 (2001).
- Giot L, Bader JS, Brouwer C *et al.*: A protein interaction map of *Drosophila melanogaster*. *Science* 302(5651), 1727–1736 (2003).
- International HapMap Consortium: A haplotype map of the human genome. *Nature* 437(7063), 1299–1320 (2005).
- Winzler EA, Shoemaker DD, Astromoff A *et al.*: Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285(5429), 901–906 (1999).
- Krogan NJ, Cagney G, Yu H *et al.*: Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440(7084), 637–643 (2006).
- Gavin AC, Aloy P, Grandi P *et al.*: Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440(7084), 631–636 (2006).
- Jansen RC, Nap JP: Genetical genomics: The added value from segregation. *Trends Genet.* 17(7), 388–391 (2001).
- Perlstein EO, Ruderfer DM, Roberts DC, Schreiber SL, Kruglyak L: Genetic basis of individual differences in the response to small-molecule drugs in yeast. *Nat. Genet.* 39(4), 496–502 (2007).
- Gatbonton T, Imbesi M, Nelson M *et al.*: Telomere length as a quantitative trait: genome-wide survey and genetic mapping of telomere length-control genes in yeast. *PLoS Genet.* 2(3), E35 (2006).
- Nogami S, Ohya Y, Yvert G: Genetic complexity and quantitative trait loci mapping of yeast morphological traits. *PLoS Genet.* 3(2), E31 (2007).
- Brem RB, Yvert G, Clinton R, Kruglyak L: Genetic dissection of transcriptional regulation in budding yeast. *Science* 296(5568), 752–755 (2002).
- Brem RB, Kruglyak L: The landscape of genetic complexity across 5,700 gene expression traits in yeast. *Proc. Natl Acad. Sci. USA* 102(5), 1572–1577 (2005).
- Schadt EE, Monks SA, Drake TA *et al.*: Genetics of gene expression surveyed in maize, mouse and man. *Nature* 422(6929), 297–302 (2003).
- Ghazalpour A, Doss S, Sheth SS *et al.*: Genomic analysis of metabolic pathway gene expression in mice. *Genome Biol.* 6(7), R59 (2005).
- Sieberts SK, Schadt EE: Moving toward a system genetics view of disease. *Mamm. Genome* 18(6–7), 389–401 (2007).
- Lan H, Stoehr JP, Nadler ST, Schueler KL, Yandell BS, Attie AD: Dimension reduction for mapping mRNA abundance as quantitative traits. *Genetics* 164(4), 1607–1614 (2003).

21. Yvert G, Brem RB, Whittle J *et al.*: Trans-acting regulatory variation in *Saccharomyces cerevisiae* and the role of transcription factors. *Nat. Genet.* 35(1), 57–64 (2003).
22. Lan H, Chen M, Flowers JB *et al.*: Combined expression trait correlations and expression quantitative trait locus mapping. *PLoS Genet.* 2(1), E6 (2006).
23. GuhaThakurta D, Xie T, Anand M *et al.*: *cis*-regulatory variations: a study of SNPs around genes showing *cis*-linkage in segregating mouse populations. *BMC Genomics* 235 (2006).
24. Alberts R, Terpstra P, Li Y, Breitling R, Nap JP, Jansen RC: Sequence polymorphisms cause many false *cis*eQTLs. *PLoS ONE* 2, E622 (2007).
25. Doss S, Schadt EE, Drake TA, Lusis AJ: *Cis*-acting expression quantitative trait loci in mice. *Genome Res.* 15(5), 681–691 (2005).
26. Ronald J, Brem RB, Whittle J, Kruglyak L: Local regulatory variation in *Saccharomyces cerevisiae*. *PLoS Genet.* 1(2), E25 (2005).
27. Brem RB, Storey JD, Whittle J, Kruglyak L: Genetic interactions between polymorphisms that affect gene expression in yeast. *Nature* 436(7051), 701–703 (2005).
28. Storey JD, Akey JM, Kruglyak L: Multiple locus linkage analysis of genomewide expression in yeast. *PLoS Biol.* 3(8), E267 (2005).
29. Drake TA, Schadt EE, Lusis AJ: Integrating genetic and gene expression data: Application to cardiovascular and metabolic traits in mice. *Mamm. Genome* 17(6), 466–479 (2006).
30. Li H, Lu L, Manly KF *et al.*: Inferring gene transcriptional modulatory relations: A genetical genomics approach. *Hum. Mol. Genet.* 14(9), 1119–1125 (2005).
31. Bing N, Hoeschele I: Genetical genomics analysis of a yeast segregant population for transcription network inference. *Genetics* 170(2), 533–542 (2005).
32. Schadt EE, Lamb J, Yang X *et al.*: An integrative genomics approach to infer causal associations between gene expression and disease. *Nat. Genet.* 37(7), 710–717 (2005).
33. Ghazalpour A, Doss S, Zhang B *et al.*: Integrating genetic and network analysis to characterize genes related to mouse weight. *PLoS Genet.* 2(8), E130 (2006).
34. Kulp DC, Jagalur M: Causal inference of regulator-target pairs by gene mapping of expression phenotypes. *BMC Genomics* 7, 125 (2006).
35. Zhu J, Wiener MC, Zhang C *et al.*: Increasing the power to detect causal associations by combining genotypic and expression data in segregating populations. *PLoS Comput. Biol.* 3(4), E69 (2007).