PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2006) **361**, 393–401
doi:10.1098/rstb.2005.1798
Published online 1 February 2006

Finding the molecular basis of complex genetic variation in humans and mice

Richard Mott*

Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK

I survey the state of the art in complex trait analysis, including the use of new experimental and computational technologies and resources becoming available, and the challenges facing us. I also discuss how the prospects of rodent model systems compare with association mapping in humans.

Keywords: complex traits; genetic mapping; association; human genetics; mouse genetics; quantitative trait locus

1. USING STUDIES INVOLVING HUMANS TO FIND GENES ASSOCIATED WITH DISEASE

I begin with a survey of the current progress in human genetics towards finding genes implicated in complex disease such as asthma, diabetes, heart disease or cancer. Recent and more comprehensive reviews of this area may be found in Colhoun *et al.* (2003), Zondervan & Cardon (2004) and Hirschhorn & Daly (2005).

In stark contrast to the successful identification of genes responsible for numerous monogenic diseases that follow simple Mendelian genetics, progress in finding the genetic basis of complex diseases in humans has been slow. This is despite the fact that genetic effects frequently account for a large proportion (up to 50%) of the variation observed in complex disease. As most monogenic diseases are rare—only cystic fibrosis could be described as at all frequent (affecting about 0.25% of Caucasians), the impact of molecular genetic analysis on common medical conditions has been minimal. There have been limited successes with asthma (Van Eerdewegh *et al.* 2002; Allen *et al.* 2003; Zhang *et al.* 2003; Laitinen *et al.* 2004), diabetes (Altshuler *et al.* 2000; Guo *et al.* 2004) and Crohn's disease (Rioux *et al.* 2001), but it is clear that the disease-predisposing alleles identified thus far only account for a small fraction of the disease cases that should be attributable to a genetic cause.

The favoured methodology for identifying genetic variants involved in complex disease is a genetic association study comparing unrelated cases and controls (Risch 2000). The basic design of an association study is simple: in a collection of unrelated individuals, one looks for a significant correlation between the disease status and genotype across the genome. Because the patients are unrelated, there will be many historical recombination events separating each pair of individuals, and hence only genetic markers (generally single nucleotide polymorphisms, or SNPs) close to a causative DNA variant(s) will be correlated with it. In principle, by genotyping a sufficiently dense set of SNPs across the genome it should be possible to map the genes with very high precision.

Two important variables that affect the success of genetic association studies are the sample size and the number of SNPs. The sample size required depends on the effect of an individual disease locus. In a complex disease there will be multiple loci involved, each explaining only a small fraction of the disease cases, possibly acting epistatically. Initially, it was hoped that there would be at least some loci with moderate to large effects, where the relative risk (RR) that an individual has the disease given that it carries a particular allele at the locus is two or more, meaning the individual is more than twice as likely to develop the disease if it carries a disease-predisposing allele. For example, the asthma-related alleles discovered to date all have RRs greater than 2 (Cookson 2004). However, many studies powered to identify such loci have failed to detect anything; consequently it is now accepted that most RRs will be of the order of 1.5 or less. The problem is that as the RR approaches 1 (at which point there is no correlation with disease status), the sample size required to detect it increases rapidly. For example, Zondervan & Cardon 2004 and Colhoun *et al.* (2003) suggest that 5000 cases and 5000 controls will be required to detect a locus with RR 1.2–1.5. While only 1000 cases and 1000 controls are required to detect a locus with RR 2, at 80% power.

Second, the number of SNPs required for an association study depends on the pattern of linkage disequilibrium (LD) in the population. LD measures the extent to which the genotype at one SNP will be correlated with that at a nearby SNP. In the simplest case, where every SNP is typed, it can be reasonably assumed that the causative variant will be included in the study (I am ignoring the possibility that the causative variant is not in fact a SNP at all, but some more complex alteration in DNA sequence, such as a polymorphic repetitive sequence, or a combination of closely linked SNPs). However, genotyping every SNP is not currently feasible. Instead, we assume that by typing a sufficiently dense set of SNPs, the remaining ungenotyped SNPs will be in LD with those whose genotypes have been determined, and consequently the effect of all variants can be surveyed. Measures of LD

*richard.mott@well.ox.ac.uk

One contribution of 15 to a Discussion Meeting Issue 'Bioinformatics: from molecules to systems'.

tell us to what extent a SNP can be used as a surrogate for others (Forton *et al*. 2005; Ke *et al*. 2005).

Our understanding of LD has recently undergone a revolution thanks to the efforts of various large-scale collaborations, especially the International HapMap Consortium (www.hapmap.org). We now know that LD is complex and variable, and depends on the ancestry of the sampled individuals. For example, there is generally far less LD in Africans than Caucasians, most probably because at some point in their history Caucasians passed through a population bottleneck which both reduced the extent of genetic variation and introduced long-range correlation along the genome, which has only gradually decayed over subsequent generations. Furthermore, SNPs that have arisen as the result of recent mutation tend to be in LD with much larger regions of the genome than do ancient variants, so within a given region some SNPs will show significant LD with everything else whilst others will not, in an apparently unpredictable manner.

Understanding the LD structure of the genome is essential in order to pick SNPs for genotyping: one can predict the likely genotype of a SNP in an individual based on the haplotypes determined at the nearby SNPs. The assumption is that it should be possible to map all functional variants using such a strategy. Calculations suggest that a carefully selected set of about one million SNPs (sometimes called 'tagging SNPs') should enable the prediction of the genotypes at about half of other known but ungenotyped SNPs (Hirschhorn & Daly 2005). There already exist SNP microarrays with 0.65 million SNPs, so this task will be technically possible within a year or so, and while very expensive at present, genotyping costs are falling dramatically as array-based technologies become standard. As a consequence we will require methods that can analyse billions of genotypes in each study.

The above issues are largely technical in nature and can be solved, given sufficient resources and technology. However, a key assumption behind association mapping, the 'common disease–common variant hypothesis' is less easily dealt with. This hypothesis says that disease-predisposing variants will exist at relatively high frequency (i.e. greater than 1%) in the population. The variants are thought to be ancient alleles occurring on specific haplotype backgrounds. This is precisely the kind of variant that an association study using tagging SNPs will detect. The alternative hypothesis is that the disease-predisposing alleles for a given disease arose from sporadic new mutations, perhaps around the same gene, but occurring on different haplotype backgrounds. Different families with a history of the same disease would owe their condition to different mutations. They would perhaps be detectable using a family based strategy which does not assume a common origin for the disease-predisposing allele, but are much harder to detect with an association study (Pritchard 2001).

So, association studies are best at detecting commoner variants and their power to detect a variant diminishes as the allele frequency of the causative variant drops. Even an association study based on one million tagging SNPs and 10 000 individuals would probably fail if the allele frequency falls to less than 0.5%. There are analytical methods that allow for multiple founder events (Morris *et al*. 2002) but the power to detect association will still be low. The best solution would be to resequence the genomes of the cases and controls. At present this is impossible, but technological developments should make at least partial resequencing feasible within 5 years.

The Wellcome Trust Case Control Consortium is in the process of performing genome-wide association studies over a range of diseases (including type I and type II diabetes, rheumatoid arthritis, susceptibility to TB and bipolar depression), genotyping 1000 cases per disease and 3000 shared controls across 0.65 million SNPs, with an additional 1000 cases per disease for genotyping SNPs identified as promising candidates. The results of this experiment are keenly awaited and will help answer some of the points raised here.

Therefore, over the next 2–3 years we can expect an answer to the question 'do genetic association studies find complex disease alleles?' If positive, we will have gained a much deeper understanding of the etiology of complex disease, which should eventually lead to improved treatments. Success here can be defined in two ways. The stronger goal is to find the major genetic determinants of complex disease, so that one could predict with accuracy the likelihood that an individual will develop a given condition, based on their DNA. A weaker goal, applicable if the common disease–common variant hypothesis does not hold, is to find alleles which may only explain a small proportion of disease cases, but whose discovery leads to a deeper understanding of the disease, and hence to possible therapies. It is possible that, while we may not be able to do the former, we may succeed with the latter.

If unsuccessful, we will need to develop other tools. In the longer term, a number of BIOBANK projects (see http://www.ukbiobank.ac.uk) are being set up in which very large cohorts (over 10 000) will be recruited and followed up over many years. These studies will be large enough to detect genetic effects with small RRs, but will take many years to produce results.

2. ANIMAL MODELS

What alternatives are available? The rest of this paper describes the use of rodents, and particularly mice, to map disease genes. We will see that while many of the same problems that affect studies in humans are present in animal studies, the latter have several unique aspects, which make their use very attractive. A fuller review of quantitative trait mapping in rodents is given by Flint *et al*. (2005).

While disease mapping in humans is usually framed in terms of dichotomous outcomes (the disease is absent or present), genetic mapping in rodents more often deals with quantitative phenotypes. From a statistical point of view, the difference is minor. Quantitative phenotypes are modelled in a multiple linear regression framework, which takes into account genetic and environmental factors, together with gene–gene and gene–environment interactions. Dichotomous phenotypes are modelled in a logistic multiple

regression framework, which is identical to the linear regression case except that the analysis models the probability of being a disease case. Therefore in this discussion a complex phenotype can be either quantitative or dichotomous.

The first requirement for the successful use of animal models is that the model is relevant to a given human phenotype. Many such models now exist, sometimes made by knocking out the mouse gene orthologous to a known disease gene in humans, or by making a transgenic animal containing the human disease gene (e.g. for Huntington's disease; Cha *et al.* 1998). It is remarkable that, in general, the mouse knockout will show a phenotype change related to the human disease. This suggests the underlying gene networks for humans and rodents have similar weak spots, and therefore similar structures.

Other models have been made by selective breeding, e.g. the non-obese diabetic strain of mice (Makino *et al.* 1980). In other cases the model comprises an assay that can be applied to any mouse. For example there are numerous behavioural tests designed to measure anxiety. One, the Open Field Test, comprises a brightly lit arena in which a mouse in introduced and tracked for five minutes. Since mice avoid being out in the open in daylight this environment is potentially threatening to the animal and therefore anxiogenic. The distance travelled and the amount of excrement (number of fecal boli) produced are recorded. An anxious mouse is defined to have low activity and produce many boli. It might be thought difficult to be certain that a mouse is anxious, but if the animals are given a drug used to treat anxiety in humans then their behaviour is modified in the test, and in the expected direction, suggesting that this (and other similar) tests do measure anxiety-related behaviour.

Although knockouts and transgenes are very useful models, it is obviously hard to make a knockout if one does not know which gene to target. Random *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screens (Quwailid *et al.* 2004) have been set-up to generate mutants and then screen them against a battery of phenotypes, but these are only practical if the phenotype is so extreme that is can be detected by observing a single animal, and the mutation must be dominant. Complex diseases do not fall into this category, and there would be a high probability that even if the right gene was knocked out, the affected animal's phenotype would only differ marginally from the wild-type; tens or hundreds of clones of the same animal would have to be tested to see an effect, which is not feasible when they are generated randomly.

The International Mouse Knockout Consortium (Austin *et al.* 2004) proposes a systematic programme to make a knockout mouse for each of the *ca* 28 000 mouse genes. Currently, there exist mouse knockouts for about 10% of known genes, but they are distributed across many laboratories and not all are freely available. This ambitious project, should it be funded, would make a valuable contribution, but is subject to the same problems as ENU screens—it is still not feasible to test all knockouts against a given phenotype.

3. QUANTITATIVE TRAIT LOCUS MAPPING

As well as studying induced mutations it is possible to examine the effects of naturally occurring variation on the phenotype. The existence of reproducible phenotypic differences between inbred strains of mice has for long been used to demonstrate the importance of genetic effects on complex phenotypes, including those that model human disease. Many of these are documented in the Mouse Phenome database (http://www.jax.org/phenome). It is on the basis of this observation that the highly successful field of quantitative trait locus (QTL) mapping is based. Here, a quantitative trait is any phenotype measured on a numerical scale. Most phenotypes can be cast into a numeric form, and so are suitable for QTL analysis. For example, we define a phenotype called EMO (for emotionality) from the Open Field Test, as the difference between the standardized distance and the standardized number of faeces produced during a five-minute observation period.

One standard design for QTL mapping is the F_2 intercross. Two inbred strains, A and B, are crossed to produce F_1 progeny. All the pairs of homologous chromosomes comprise one A and one B chromosome. The F_1 are then intercrossed to produce an F_2 generation. Meiosis will ensure that each chromosome is a mixture of A and B, with roughly one cross over per chromosome. About 200 F_2 animals are phenotyped, and their DNA genotyped using around 100 markers distributed evenly across the genome. The approximate locations of quantitative trait loci are determined by looking for association between the phenotype and the marker's genotypes. The F_2 intercross is simple yet extraordinarily powerful at detecting QTL. Over 2000 QTL for a wide variety of traits are recorded in the mouse genome informatics (MGI) database (www.informatics.jax.org).

The difficulty with this experimental design is the lack of mapping resolution. By increasing the number of animals, it is possible to increase resolution but tens of thousands of animals would be required to obtain mapping resolution down to a single gene, which is the ideal.

In fact, the problem of how to narrow QTL intervals is a major challenge, analogous to the problem in human genetics of proceeding from a broad linkage peak to the gene. In only a handful of cases has the responsible gene been cloned, and moreover these cases are all very unusual in that they involve a QTL explaining over 15% of the total phenotypic variance. The average detectable QTL explains about 5% of the variance. Hence, we need high resolution mapping methods that work for small-effect QTL. This problem has now become very serious; we are detecting large numbers of QTL but cloning hardly any genes (Flint *et al.* 2005).

The source of the difficulty is that each F_2 chromosome only contains about one recombination event. Adding recombination by intercrossing for more generations—the Advanced Intercross (AI)—is one solution (Darvasi & Soller 1995). AI mice have genomes that are a finer-grained mosaic of the two founders, A, B, say. The degree of mosaicism increases with the number of generations in the intercross.

A generalization of the AI is the Heterogeneous Stock (HS; McClearn *et al.* 1970). HS are descended from eight founder strains, which are mixed together by a series of intercrosses, and then the population is maintained in a semi-random mating scheme for many generations until the genomes are random mosaics of all eight founders. The advantage of the HS over the AI is the increased genetic diversity. However, mapping in an HS is more complicated than an AI because there are eight possible haplotypes at each locus, and so single marker association mapping does not work well; most markers cannot distinguish all eight strains and of course diallelic SNPs only distinguish two. Consequently, if a QTL is caused by a variant which separates the four strains A, B, C, D from the remaining four E, F, G, H but the nearest markers happen to distinguish A, B, G, H from C, D, E, F, then there will be no power to detect the trait locus using single-marker association.

The solution is to perform a multipoint analysis where more than one marker is considered at a time (Mott *et al.* 2000). The data can be thought of as a hidden Markov model, in which the observed data are the genotypes at a sequence of markers, and the hidden states are the underlying founder strains. The objective is to calculate the probability that an individual is descended from a particular pair of founders at a given locus. We use these descent probabilities to estimate the phenotypic effect attributable to each founder at the locus. If these effects are significantly different from one another then there is evidence for a QTL.

The mosaic structure of AI and HS means that much greater mapping resolution is possible, provided a sufficiently dense set of markers (200–300 kb apart) is used. Mapping accuracy to under a centiMorgan is achievable, and although this is much better than the typical F_2 resolution of 20 cM, it is not sufficient to identify a single gene. There are, on average 10 known genes per Mbp in the mouse and 1 cM is approximately 2 Mbp, although the mapping between physical and genetic distance is highly variable, with the presence of recombination hotspots and cold spots. Therefore, we must expect that HS mapping will only provide us with a menu of genes for further analysis. For this task one must either systematically examine each gene, a very arduous task, or move to a system with an even higher density of recombinants.

The key is to use a population of mice that have been maintained in a large mating population for many generations. HS are typically maintained in a population of 40–50 mating pairs. Computer simulations suggest that if an HS were maintained with 100 pairs then mapping resolution would be much greater. However, since this takes time and money, we investigated the potential for gene mapping of a standard commercial outbred population called MF1. The ancestry of MF1 is not known for certain, but we were able to show by sequencing that the MF1 genomes closely resemble an HS but with a much higher degree of recombination. Hence they could be analysed in the same way, and we were able to map a QTL for behaviour to a resolution of about 100 kb (Yalcin *et al.* 2004*b*).

Use of outbred populations like MF_1 promises much. However, it is important to demonstrate first that their genomes really are mosaics of other standard strains. The issue becomes even more important with so-called *in silico* mapping.

In silico mapping (Grupe *et al.* 2001; Pletcher *et al.* 2004) uses a panel of standard laboratory strains to map QTL. The idea is to measure the phenotype on each strain, using replicates if necessary to reduce the variance attributable to experimental error. The strains are genotyped across the genome using a dense set of markers. QTLs correspond to loci where the strain distribution patterns of genotypes and phenotypes are correlated. Strong claims have been made for *in silico* mapping (Grupe *et al.* 2001), which have been contested by others (Darvasi 2001). One key question depends on the ancestry of the inbred strains and the effect it has on the pattern of haplotype sharing. The assumption behind *in silico* mapping is that the genomes of the inbreds are ultra-fine mosaics of a smaller number of shared haplotypes. Consequently by genotyping markers at sufficient density one can infer the genotypes at any intermediate ungenotyped variant with high certainty.

This assumption is the mouse equivalent of the human common disease–common variant hypothesis. Is it true? Studies where inbreds have been resequenced at a number of loci (Frazer *et al.* 2004; Yalcin *et al.* 2004*a*) indicate that, rather than sharing haplotypes, it is better to think of the relationship between inbreds as a mosaic of phylogenetic trees rather than haplotypes. Locally, almost all variants are consistent with a particular tree, i.e. the strain distribution pattern of the variant is consistent with a single mutation arising on the tree. Variants with the different strain distributions coexist on the same tree, giving rise to patterns, which can appear random when the data are analysed for haplotype sharing. The tree topology changes along the genome. It is not yet clear how variable the tree is, but this will be known soon now that dense genotype sets of inbreds have become available (see e.g. http://www.well.ox.ac.uk/mouse/INBREDS/).

A second question is how many distinct strains are required for *in silico* mapping. There is no consensus on this question, with estimates for the minimum number of useful strains ranging from 8 to 100. With a smaller number of strains there is a greater chance that 'ghost' QTL may occur, where by chance unlinked loci have very similar strain distribution patterns yet only one contains the functional variant. Further, the mapping resolution should be less with a smaller number of strains. *In silico* mapping appears to be most useful in conjunction with standard QTL mapping strategies such as the F_2 cross, where it can be used to refine a QTL detected in the cross, for instance by identifying regions of the genome identical between mouse strains, and which therefore cannot contain a functional variant (Cervino *et al.* 2005).

QTL mapping using Recombinant Inbred Lines (RIL) is superficially like *in silico* mapping, but RIL are uncontroversial. RIL are made by intercrossing two inbred lines to make a standard F_2 population, then breeding a number of inbred lines by repeated brother–sister matings for about 20 generations. The resulting

lines are mosaics of the two founder strains. They can be used for QTL mapping using the *in silico* method. The difference is that with RIL there is no doubt as to the ancestry of the strains; one knows for certain only two haplotypes are present so one can interpolate between markers with great certainty. One cannot do this with the standard inbred strains. Ideally, for *in silico* mapping one needs to resequence the strains. Therefore it is especially welcome that the National Institutes of Environmental Health Sciences has begun partial resequencing of 15 common strains (http://www.niehs.nih.gov/oc/news/micedna.htm) using microarrays. The cost of resequencing is falling with new technologies, so it is likely that all standard strains a will be resequenced eventually.

The problem with current RIL panels is their relatively poor mapping resolution, approximately equivalent to an F_2 intercross. By contrast, if *in silico* mapping can be made to work reliably, it promises much higher resolution because of the higher density of recombinants. Again, the comparison with human genetics is helpful—showing that standard mouse strains are in effect mosaics of each other is equivalent to showing the haplotypes found in humans are mosaics of a small number of blocks. The evidence thus far suggests that the haplotype block view of the mouse and human genomes is not the complete picture, although it captures some of the structure.

The Complex Trait Consortium (http://www.complextrait.org) has proposed making a new panel of 1000 RIL, descended from eight founder strains (Churchill *et al.* 2004), somewhat like the HS. Simulations indicate that one advantage of this panel would be much improved mapping resolution—down to 0.5 cM (approx. 1 Mb)—although even this resource would not deliver single gene resolution, for which one requires a resolution of about 100 kb.

4. CONGENICS AND CONSOMICS

Congenics are still a mainstay of fine-mapping QTLs in rodents. By repeatedly backcrossing one strain onto another, it is possible to create animals with a genomic region that contains a QTL from one strain and the remainder of their genome from the other; subsequent intercrossing makes the genomic segment homozygous and the mouse fully inbred. Congenics are used to refine an interval containing a QTL until, in theory, it only contains a single gene. However, inadequate or incorrect assumptions about the distribution of chromosome segments, the population structure, the marker spacing and the selection strategy may mean that the breeding does not go as predicted (Visscher 1999). Moreover, attempts to refine QTL identified in (say) F_2 crosses have frequently shown the QTL split into sub-QTL. There are now a large number of examples of this phenomenon, including QTLs influencing seizures (Legare *et al.* 2000), obesity (Stylianou *et al.* 2004), growth (Christians & Keightley 2004), blood pressure (Frantz *et al.* 2001; Alemayehu *et al.* 2002; Garrett & Rapp 2002*a*,*b*; Ariyarajah *et al.* 2004), diabetes (Podolin *et al.* 1998), antibody production (Puel *et al.* 1998) and infection (Bihl *et al.* 1999).

Consomics, also called chromosome substitution strains (CSS), are congenics where one entire chromosome is derived from one strain and the remainder from another. QTL mapping occurs by the relatively simple process of comparing the phenotypes of each strain with the parental background strain. CSS were first used to map QTL in mice in 1999 (Matin *et al.* 1999); theoretical aspects were described four years ago for mice (Nadeau *et al.* 2000), and more recently for rats (Roman *et al.* 2002; Cowley *et al.* 2004). The method has a long history in plant (Law 1966) and *Drosophila* genetics (Caligari & Mather 1975). The first complete CSS set, created from A/J and C57BL/6 strains, was produced earlier this year and used to detect QTLs across the mouse genome (Singer *et al.* 2004, 2005).

The ease of QTL detection using CSS follows from two features: first, the background genetic variance is reduced so each QTL explains a greater proportion of the total phenotypic variation. Second, a lower significance level is needed for QTL detection because, compared to the 100 odd markers tested in an F_2, only 21 comparisons need be made. Singer and colleagues point out that the F_2 intercross requires at least 35% more animals for QTL detection (Singer *et al.* 2004, 2005). Nevertheless, Belknap estimates that to detect a 6% QTL with 50% power will need 20 CSS and 20 parental animals for each comparison; or between 3 and 400 animals (depending on how many background animals are used) for a genome scan (Belknap 2003). That number agrees with the actual figure of 435 animals used to map QTLs in the first mouse CSS experiment (Singer *et al.* 2004, 2005). Note that this is not a substantial saving on the numbers used for an F_2 intercross.

A comparison between parental and CSS strains will only map a QTL to a chromosome. For higher resolution mapping, CSS allow the rapid creation of a congenic, either by interval specific congenic strains or recombinant progeny testing (Darvasi 1998). Because of the relative increase in effect size, congenic construction and recombinant progeny testing will be easier and require 3–4 generations to reduce the interval to 1 cM, rather than the 9–10 generations required when starting from an F_2 intercross (Belknap 2003).

QTL mapping in a CSS delivers researchers faster to the same, point that classical strategies have led them, but no further. The main drawback of the method is that it makes no allowances for the fractionation of a large QTL effect into many smaller effect loci. This is the problem that has for so long beset the use of congenics for QTL dissection and gene identification. CSS mapping is a powerful method for the identification of small effect QTL, but it does not offer advantages over other methods for the identification of genes.

5. QUANTITATIVE COMPLEMENTATION

How do we confirm that a gene is a quantitative trait gene (QTG)? At best, QTL analysis finds regions which will contain only a handful of genes, and at worst, several hundred. It has proved very difficult to

move from QTL to gene unless the QTL contains only one gene.

To make matters worse, there is no consensus on how much proof is required, although proposals have been made (Abiola *et al.* 2003). In Mendelian diseases, it is often the case that the identified variant also disrupts gene function, either by deleting part of a gene or causing premature termination of the transcript, and so the problem is non-existent. For complex traits the causal variant may lie outside of the gene in a control element, and have a subtle effect on gene expression or on splicing. Therefore, the end game in complex disease mapping is doubly hard: there may be several variants with equal claim to be the quantitative trait nucleotide (indeed there may be more than one) and it is not clear on which gene(s) the variant(s) acts. It is common for a QTL identified as a broad peak in an F_2 intercross to fractionate into several smaller peaks when mapped at higher resolution. The effect attributable to each sub-QTL will be smaller which makes the mapping harder.

The gold standard would be to show that mutating a single nucleotide on an otherwise unchanged genetic background causes the change in phenotype, but chromosomal engineering is extremely time-consuming and expensive. Instead, a number of other criteria have been adopted. A list of 20 'cloned' quantitative genes is listed in (Flint *et al.* 2005). The weight of evidence in favour of each gene varies considerably. For instance, Ferraro and colleagues propose *kcnj10* as the gene at a seizure susceptibility locus on the basis of finding sequence variants in coding regions and gene expression in the relevant tissue, but have to consider data for 120 genes within the critical region (Ferraro *et al.* 2004); Shirley and colleagues apply similar criteria for the candidacy of *Mpdz*, but have a much smaller interval, with only three known and three predicted genes to validate, and so are able to make a more convincing case for gene identification (Shirley *et al.* 2004).

Here we discuss one promising method called quantitative complementation (QC) to test if a gene is functional, and which is applicable to any gene. QC originated in work on the fruit fly *Drosophila* (Long *et al.* 1996). The idea is to look for an interaction between the gene to be tested and the variant causing the trait variation. It does not require one to know where the variant is on the genome.

The experimental design requires the offspring from four crosses. An inbred animal bearing one QTL allele (for example 'high') is mated to an inbred animal with a null allele of the gene of interest ('m') and also to the co-isogenic wild-type animal ('wt'). A similar pair of crosses is established, but this time using an inbred strain with the alternative QTL allele ('low'). If the difference in mean phenotype between the high/m and low/m genotypes is greater than that between the high/wt and low/wt genotypes then we have evidence of quantitative failure of the mutation to complement the QTL alleles. This is detected as a statistical 'Cross' (m or wt) by 'Line' (high or low) interaction in a two-way analysis of variance.

One interpretation of a significant interaction is that the expression of the wt (i.e. functional) gene is modulated by a QTL allele on the homologous chromosome. It should be noted that the test does not implicate any particular QTL, which could be anywhere on the genome where the high and low strains differ. Furthermore, a negative result is not conclusive, since it could mean either the gene is not under the control of a QTL or that the gene is under the control of a nearby QTL allele on the same chromosome.

QC tests need co-isogenic wild-types, which can be difficult to obtain in mice. Knockouts created in a 129 strain are usually backcrossed onto a different strain (typically C57BL/6) so that often no pure co-isogenic wild-type is available. However when the experimenter has only the hybrid to work with, the problem of mixed background can sometimes be overcome by taking advantage of the mosaic nature of the mouse genome: some regions of the 129 strain will be identical to the strain onto which it has been backcrossed. Where the targeted region occurs in such a region (or in a region which is known from genetic crosses not to carry QTLs that influence the trait of interest), and the rest of the 129 strain has been removed by repeated backcrossing, then it should be possible to find an appropriate co-isogenic wild-type. For example, by extensive re-sequencing of the 129, C57BL/6J and DBA/2J strains, we showed that inbred C57BL/6 could be combined with a targeted mutation of the *rgs2* gene in a QC test (Yalcin *et al.* 2004*b*). This arduous task can be avoided in the future once the relevant strains have been fully resequenced. Alternatively, knockouts could be made and maintained on a single background or obtained by screening the DNA of mutagenized inbred mice (Coghill *et al.* 1999).

6. DISCUSSION

The great strength of genetic mapping is that it treats the organism as a black box: the method seeks to find those DNA variants that are highly correlated with the trait variation. No assumptions are made about the biology that links the DNA to the trait. Therefore, it is ideal for the genetic dissection of complicated traits such as behaviour. However, viewed from another standpoint this is also the method's weakness—it may tell us which variants are functional, but not how. In fact it need not even tell us which genes are involved, since the functional variant may be distant from the gene on which it acts, and even lie within the intron of another gene.

Unfortunately our current understanding of gene function is too limited to let us predict *a priori* which genes are involved with a particular trait, and educated guesses based on gene expression or other functional data are not much better. While the state of genome annotation could definitely be improved, even if all genes were fully annotated using all the information in the published literature, our knowledge would still be incomplete.

One problem is that it is misleading to think of a gene as being *for* something; in reality many genes will influence a trait, and a single gene will influence many traits.

Systems biology may be defined as the study of the web of gene interactions, and is a better way to think about gene function. However, current studies using gene expression and protein interaction data, while a leap forward, are still incomplete and approximate. It is not yet clear how to integrate all the data together to produce a model of the cellular machine.

There has been some debate on the best strategy to dissect gene networks. At the risk of painting a caricature, protagonists of the old-fashioned gene-by-gene approach would maintain that it is better to know a small number of big truths than a large number of small ones. Therefore, it is better first to identify individual genes of large effect in a genome scan and then work into the network by looking for interactions with those genes, and so on. The alternative view embraces complexity, and looks for clusters of genes with correlated patterns of expression, ignoring the individual effects of genes on the phenotype. The task then is to determine which genes are causal to the phenotype and which are merely correlated with it (Ghazalpour *et al.* 2005; Schadt *et al.* 2005).

Can genetics help? One way to predict locus–locus interactions might be to search for epistasis, by which we mean two loci whose joint effect on the trait is significantly different from the sum of the individual locus effects. Recent studies have indicated that epistasis may be common, particularly in developmental traits (Carlborg & Haley 2004), although there is less evidence for epistasis in behaviour (Flint *et al.* 2004).

Another recent development has been the use of RIL panels to map QTL where the phenotype is the expression level of a gene (called an eQTL), determined from a series of microarray experiments applied across the RIL panel. There is an eQTL that maps close (given the achievable mapping resolution) to the corresponding gene in about 60% of the genes surveyed (Doss *et al.* 2005). However, there are also a number of loci that affect the expression of many genes. These are most likely transcription factors. Thus one can begin to construct a gene interaction network from this type of experiment (Chesler *et al.* 2005).

In his Nobel Lecture Sydney Brenner (2003) wrote:

> My second *gedanken* project is called **Humanity's Genes.** It arose in my mind during a discussion of a proposal to take the inbred lines of mice, and extensively intercross them to generate 30,000 different mice representing different mosaics of the initial gene pools. Specially trained mouse phenotypers would then analyse the physiological properties in these mice and correlate them with their individual genomes. Unfortunately, the latter is the difficult task, as today there is no reasonable technology that can achieve this in any depth. However, suppose technology existed which made it easy to characterize 30,000 genomes, perhaps even to the point of resequencing them, would we bother to do this work with mice? We could go directly to humans, where we already have large numbers of diverse genomes, with skilled and expensively trained phenotypers, called doctors, studying them. Thus, since the technology does not exist, it now needs to be invented to provide the means of accurately analyzing large populations of genomes for detailed studies of natural human genetic variation and its correlation with phenotypes of health and disease. I believe that this will be the major challenge in human biology and medicine in the next decade. I am convinced that we will make our significant discoveries in humans and that the mouse will be used to validate the human findings by genetic synthesis, much in the same way as the chemist confirms a structure analysis by chemical synthesis.

At first sight this argument may seem persuasive, but the problems we have described that confront human association studies may limit the effectiveness of a research programme based entirely on the observation of patients under uncontrolled environmental conditions. Present efforts to integrate clinical work with human genetics (the so-called Genetics Knowledge Parks) are in their infancy. While they promise much, there are considerable ethical, technological and sociological issues to be addressed. Patients may give limited consent to permit their medical records and DNA be analysed, but it is harder to imagine that the majority of healthy individuals would be prepared to act as guinea pigs and permit the level of detailed and repeated measurement possible on animal models, in a controlled environment. On the other hand, it is now clear that very large sample sizes are required in order for association studies on humans to succeed, so we may require something of equivalent scope and ambition.

While there are no serious proposals to make 30 000 mosaic mice, there are plans underway to make up to 1000 (Churchill *et al.* 2004). The statistical methods required to analyse mosaic mice exist and have been used successfully in the analysis of HS and other outbred stocks (Mott *et al.* 2000). The Complex Trait Consortium (www.complextrait.org) has already begun breeding the first few hundred lines, which should be available for use by about the year 2010. Simulations indicate that even a modest set of 100–200 lines would be very useful for QTL mapping.

Finally, the clonal nature of inbred strains and knockouts means that experiments can be repeated under varying environmental conditions while keeping the genetics constant. This is impossible with humans. Thus it may be the case that the mouse will be the primary tool to discover the genes underlying complex disease, and candidate gene studies in humans used as a follow up to confirm the findings. It is also important to recognize that, even when a disease-predisposing allele has been found, turning this discovery into a therapy will require us to understand the underlying molecular biology. For ethical reasons it is not usually possible to use living human subjects, but requires extensive use of cell lines and animal models. Therefore the combination of comprehensive panels of mouse knockouts and mosaics will be extremely powerful in answering a wide range of medical and biological questions.

In summary, I have presented a comparison of the achievements and prospects for complex disease mapping using human and mouse genetics, emphasizing the parallels and differences. Both fields are facing difficulties when applied to complex diseases, but the mouse has a wider range of resources, despite being hampered by relatively low funding compared to

human genetics. Most probably a combined approach of mouse genetics and transcriptomics together with studies involving humans will yield the fastest progress. The point is that the tools to do this work are either available now or under development. All that is required is the vision, cooperation, energy and money to use them wisely.

This work was funded by a grant from the Wellcome Trust. I thank Jonathan Flint for helpful comments on the manuscript.

REFERENCES

Abiola, O. *et al.* 2003 The nature and identification of quantitative trait loci: a community's view. *Nat. Rev. Genet.* **4**, 911–916.

Alemayehu, A., Breen, L., Krenova, D. & Printz, M. P. 2002 Reciprocal rat chromosome 2 congenic strains reveal contrasting blood pressure and heart rate QTL. *Physiol. Genomics* **10**, 199–210.

Allen, M. *et al.* 2003 Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat. Genet.* **35**, 258–263. (doi:10.1038/ng1256)

Altshuler, D. *et al.* 2000 The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat. Genet.* **26**, 76–80. (doi:10.1038/79839)

Ariyarajah, A., Palijan, A., Dutil, J., Prithiviraj, K., Deng, Y. & Deng, A. Y. 2004 Dissecting quantitative trait loci into opposite blood pressure effects on Dahl rat chromosome 8 by congenic strains. *J. Hypertens* **22**, 1495–1502. (doi:10.1097/01.hjh.0000133720.94075.6f)

Austin, C. P. *et al.* 2004 The knockout mouse project. *Nat. Genet.* **36**, 921–924. (doi:10.1038/ng0904-921)

Belknap, J. K. 2003 Chromosome substitution strains: some quantitative considerations for genome scans and fine mapping. *Mamm. Genome* **14**, 723–732. (doi:10.1007/s00335-003-2264-1)

Bihl, F., Brahic, M. & Bureau, J. F. 1999 Two loci, Tmevp2 and Tmevp3, located on the telomeric region of chromosome 10, control the persistence of Theiler's virus in the central nervous system of mice. *Genetics* **152**, 385–392.

Brenner, S. 2003 Nobel lecture. Nature's gift to science. *Biosci. Rep.* **23**, 225–237.

Caligari, P. D. & Mather, K. 1975 Genotype–environment interaction. III. Interactions in *Drosophila melanogaster*. *Proc. R. Soc. B* **191**, 387–411.

Carlborg, O. & Haley, C. S. 2004 Epistasis: too often neglected in complex trait studies? *Nat. Rev. Genet.* **5**, 618–625. (doi:10.1038/nrg1407)

Cervino, A. C. *et al.* 2005 Integrating QTL and high-density SNP analyses in mice to identify Insig2 as a susceptibility gene for plasma cholesterol levels. *Genomics* **86**, 505–5017.

Cha, J. H., Kosinski, C. M., Kerner, J. A., Alsdorf, S. A., Mangiarini, L., Davies, S. W., Penney, J. B., Bates, G. P. & Young, A. B. 1998 Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. *Proc. Natl Acad. Sci. USA* **95**, 6480–6485. (doi:10.1073/pnas.95.11.6480)

Chesler, E. J. *et al.* 2005 Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. *Nat. Genet.* **37**, 233–242. (doi:10.1038/ng1518)

Christians, J. K. & Keightley, P. D. 2004 Fine mapping of a murine growth locus to a 1.4-cM region and resolution of linked QTL. *Mamm. Genome* **15**, 482–491. (doi:10.1007/s00335-004-3046-0)

Churchill, G. A. *et al.* 2004 The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat. Genet.* **36**, 1133–1137. (doi:10.1038/ng1104-1133)

Coghill, E. L., Hughill, A., Parkinson, N., Davison, C., Glenister, P., Clements, S., Hunter, J., Cox, R. D. & Brown, S. D. M. 1999 A gene driven approach to the identification of ENU mutants in the mouse. *Nat. Genet.* **30**, 255–256. (doi:10.1038/ng847)

Colhoun, H. M., McKeigue, P. M. & Davey Smith, G. 2003 Problems of reporting genetic associations with complex outcomes. *Lancet* **361**, 865–872. (doi:10.1016/S0140-6736(03)12715-8)

Cookson, W. 2004 The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat. Rev. Immunol.* **4**, 978–988. (doi:10.1038/nri1500)

Cowley Jr, A. W., Liang, M., Roman, R. J., Greene, A. S. & Jacob, H. J. 2004 Consomic rat model systems for physiological genomics. *Acta Physiol. Scand.* **181**, 585–592. (doi:10.1111/j.1365-201X.2004.01334.x)

Darvasi, A. 1998 Experimental strategies for the genetic dissection of complex traits in animal models. *Nat. Genet.* **18**, 19–24. (doi:10.1038/ng0198-19)

Darvasi, A. 2001 *In silico* mapping of mouse quantitative trait loci. *Science* **294**, 2423. (doi:10.1126/science.294.5551.2423a)

Darvasi, A. & Soller, M. 1995 Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199–1207.

Doss, S., Schadt, E. E., Drake, T. A. & Lusis, A. J. 2005 Cis-acting expression quantitative trait loci in mice. *Genome Res.* **15**, 681–691. (doi:10.1101/gr.3216905)

Ferraro, T. N. *et al.* 2004 Fine mapping of a seizure susceptibility locus on mouse Chromosome 1: nomination of Kcnj10 as a causative gene. *Mamm. Genome* **15**, 239–251. (doi:10.1007/s00335-003-2270-3)

Flint, J., De Fries, J. C. & Henderson, N. D. 2004 Little epistasis for anxiety-related measures in the DeFries strains of laboratory mice. *Mamm. Genome* **15**, 77–82. (doi:10.1007/s00335-003-3033-x)

Flint, J., Valdar, W., Shifman, S. & Mott, R. 2005 Strategies for mapping and cloning quantitative trait genes in rodents. *Nat. Rev. Genet.* **6**, 271–286. (doi:10.1038/nrg1576)

Forton, J., Kwiatkowski, D., Rockett, K., Luoni, G., Kimber, M. & Hull, J. 2005 Accuracy of haplotype reconstruction from haplotype-tagging single-nucleotide polymorphisms. *Am. J. Hum. Genet.* **76**, 438–448. (doi:10.1086/428439)

Frantz, S., Clemitson, J. R., Bihoreau, M. T., Gauguier, D. & Samani, N. J. 2001 Genetic dissection of region around the Sa gene on rat chromosome 1: evidence for multiple loci affecting blood pressure. *Hypertension* **38**, 216–221.

Frazer, K. A., Wade, C. M., Hinds, D. A., Patil, N., Cox, D. R. & Daly, M. J. 2004 Segmental phylogenetic relationships of inbred mouse strains revealed by fine-scale analysis of sequence variation across 4.6 mb of mouse genome. *Genome Res.* **14**, 1493–1500. (doi:10.1101/gr.2627804)

Garrett, M. R. & Rapp, J. P. 2002*a* Multiple blood pressure QTL on rat Chromosome 2 defined by congenic Dahl rats. *Mamm. Genome* **13**, 41–44. (doi:10.1007/s00335-001-2114-y)

Garrett, M. R. & Rapp, J. P. 2002*b* Two closely linked interactive blood pressure QTL on rat Chromosome 5 defined using congenic Dahl rats. *Physiol. Genomics* **8**, 81–86.

Ghazalpour, A., Doss, S., Sheth, S. S., Ingram-Drake, L. A., Schadt, E. E., Lusis, A. J. & Drake, T. A. 2005 Genomic analysis of metabolic pathway gene expression in mice. *Genome Biol.* **6**, R59. (doi:10.1186/gb-2005-6-7-r59)

Grupe, A., Germer, S., Usuka, J., Aud, D., Belknap, J. K., Klein, R. F., Ahluwalia, M. K., Higuchi, R. & Peltz, G. 2001 In silico mapping of complex disease-related traits in mice. *Science* **292**, 1915–1918. (doi:10.1126/science.1058889)

Guo, D. *et al.* 2004 A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat. Genet.* **36**, 837–841. (doi:10.1038/ng1391)

Hirschhorn, J. N. & Daly, M. J. 2005 Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.* **6**, 95–108. (doi:10.1038/nrg1521)

Ke, X., Miretti, M. M., Broxholme, J., Hunt, S., Beck, S., Bentley, D. R., Deloukas, P. & Cardon, L. R. 2005 A comparison of tagging methods and their tagging space. *Hum. Mol. Genet.* **14**, 2757–2767.

Laitinen, T. *et al.* 2004 Characterization of a common susceptibility locus for asthma-related traits. *Science* **304**, 300–304. (doi:10.1126/science.1090010)

Law, C. N. 1966 The location of genetic factors affecting a quantitative characters in wheat. *Genetics* **53**, 487–498.

Legare, M. E., Bartlett, F. S. & Frankel, W. N. 2000 A major effect QTL determined by multiple genes in epileptic EL mice. *Genome Res.* **10**, 42–48.

Long, A. D., Mullaney, S. L., Mackay, T. F. C. & Langley, C. H. 1996 Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. *Genetics* **144**, 1497–1510.

Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K. & Tochino, Y. 1980 Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu* **29**, 1–13.

Matin, A., Collin, G. B., Asada, Y., Varnum, D. & Nadeau, J. H. 1999 Susceptibility to testicular germ-cell tumours in a 129.MOLF-Chr 19 chromosome sustitution strain. *Nat. Genet.* **23**, 237–240. (doi:10.1038/13874)

McClearn, G. E., Wilson, J. R. & Meredith, W. 1970 The use of isogenic and heterogenic mouse stocks in behavioral research. In *Contributions to behavior-genetic analysis: the mouse as a prototype* (ed. G. Lindzey & D. Thiessen), pp. 3–22. New York: Appleton Century Crofts.

Morris, A. P., Whittaker, J. C. & Balding, D. J. 2002 Fine-scale mapping of disease loci via shattered coalescent modeling of genealogies. *Am. J. Hum. Genet.* **70**, 686–707. (doi:10.1086/339271)

Mott, R., Talbot, C. J., Turri, M. G., Collins, A. C. & Flint, J. 2000 A method for fine mapping quantitative trait loci in outbred animal stocks. *Proc. Natl Acad. Sci. USA* **97**, 12 649–12 654. (doi:10.1073/pnas.230304397)

Nadeau, J. H., Singer, J. B., Matin, A. & Lander, E. S. 2000 Analysing complex genetic traits with chromosome substitution strains. *Nat. Genet.* **24**, 221–225. (doi:10.1038/73427)

Pletcher, M. T. *et al.* 2004 Use of a dense single nucleotide polymorphism map for in silico mapping in the mouse. *PLoS Biol.* **2**, e393. (doi:10.1371/journal.pbio.0020393)

Podolin, P. L. *et al.* 1998 Localization of two insulin-dependent diabetes (Idd) genes to the Idd10 region on mouse Chromosome 3. *Mamm. Genome* **9**, 283–286. (doi:10.1007/s003359900749)

Pritchard, J. K. 2001 Are rare variants responsible for susceptibility to complex diseases? *Am. J. Hum. Genet.* **69**, 124–137. (doi:10.1086/321272)

Puel, A., Mevel, J. C., Bouthillier, Y., Decreusefond, C., Fridman, W. H., Feingold, N. & Mouton, D. 1998 Identification of two quantitative trait loci involved in antibody production on mouse chromosome 8. *Immunogenetics* **47**, 326–331. (doi:10.1007/s002510050365)

Quwailid, M. M. *et al.* 2004 A gene-driven ENU-based approach to generating an allelic series in any gene. *Mamm. Genome* **15**, 585–591. (doi:10.1007/s00335-004-2379-z)

Rioux, J. D. *et al.* 2001 Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat. Genet.* **29**, 223–228. (doi:10.1038/ng1001-223)

Risch, N. J. 2000 Searching for genetic determinants in the new millennium. *Nature* **405**, 847–856. (doi:10.1038/35015718)

Roman, R. J., Cowley, A. W., Greene, A., Kwitek, A. E., Tonaellato, P. J. & Jacob, H. J. 2002 Consomic rats for the identificaiton of genes and pathways underlying cardiovascular disease. *Cold Spring Harbor Symposia on Quantitative Biology*, vol. LXVII, pp. 309–315. New York: Cold Sping Harbor Laboratory Press.

Schadt, E. E. *et al.* 2005 An integrative genomics approach to infer causal associations between gene expression and disease. *Nat. Genet.* **37**, 710–717. (doi:10.1038/ng1589)

Shirley, R. L., Walter, N. A., Reilly, M. T., Fehr, C. & Buck, K. J. 2004 Mpdz is a quantitative trait gene for drug withdrawal seizures. *Nat. Neurosci.* **7**, 699–700. (doi:10.1038/nn1271)

Singer, J. B. *et al.* 2004 Genetic dissection of complex traits with chromosome substitution strains of mice. *Science* **304**, 445–448. (doi:10.1126/science.1093139)

Singer, J. B., Hill, A. E., Nadeau, J. H. & Lander, E. S. 2005 Mapping quantitative trait loci for anxiety in chromosome substitution strains of mice. *Genetics* **169**, 855–862.

Stylianou, I. M., Christians, J. K., Keightley, P. D., Bunger, L., Clinton, M., Bulfield, G. & Horvat, S. 2004 Genetic complexity of an obesity QTL (Fob3) revealed by detailed genetic mapping. *Mamm. Genome* **15**, 472–481. (doi:10.1007/s00335-004-3039-z)

Van Eerdewegh, P. *et al.* 2002 Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* **418**, 426–430. (doi:10.1038/nature00878)

Visscher, P. M. 1999 Speed congenics: accelerated genome recovery using genetic markers. *Genet. Res.* **74**, 81–85. (doi:10.1017/S0016672399003857)

Yalcin, B. *et al.* 2004*a* Unexpected complexity in the haplotypes of commonly used inbred strains of laboratory mice. *Proc. Natl Acad. Sci. USA* **101**, 9734–9739. (doi:10.1073/pnas.0401189101)

Yalcin, B. *et al.* 2004*b* Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. *Nat. Genet.* **36**, 1197–1202. (doi:10.1038/ng1450)

Zhang, Y. *et al.* 2003 Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat. Genet.* **34**, 181–186. (doi:10.1038/ng1153)

Zondervan, K. T. & Cardon, L. R. 2004 The complex interplay among factors that influence allelic association. *Nat. Rev. Genet.* **5**, 89–100. (doi:10.1038/ nrg1270)