Genome-wide association studies of therapeutic response: addressing the complexities

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“The stability and measurability of genetic variants make them very attractive as potential predictive biomarkers.”

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Genome-wide association studies (GWAS) have led to the discovery of thousands of genetic variants associated with disease susceptibility or with diverse quantitative traits. The catalog of GWAS curated by the National Human Genome Research Institute and the European Bioinformatics Institute lists 3675 publications and 87,081 unique associations (26 November 2018 release) [1]. Early studies with small sample sizes (most notably the 2005 GWAS of age-related macular degeneration [2], which was based on only 96 cases and 50 controls) were successful in identifying variants of large effect, with per-allele odds ratios of over 2. However, in view of the multiple testing inherent in GWAS, it has only been possible to discover variants of small effect through the accumulation of very large datasets, sometimes in the tens of thousands. These polygenic effects, although not individually highly predictive of outcome, can be combined as genetic risk scores which have greater predictive potential. The ability to investigate the entire genome without prior hypotheses can also lead to the discovery of unexpected trait-related pathways.

Discovering the genetic basis of disease or of quantitative human traits has many potential benefits, but such knowledge is often not immediately actionable. In contrast, the clinical utility of predicting drug response is evident, particularly where alternative therapies are available or where there is a severe adverse reaction to be avoided. Clearly, if the success of GWAS in other contexts could be replicated in drug response (*Pharmacogenomics*), this would be of enormous benefit to patients.

To date, success in pharmacogenomics has been more limited than for other GWAS. Only a small minority (<10%) of GWAS have been in this area, and most have been based on sample sizes of less than 1000 fully genotyped subjects [3]. There are, however, notable important successes, for example in relation to warfarin dosage. Ethnicity-specific algorithms are in place that make use of genetic information on *CYP2C9*, *VKORC1*, *CYP4F2* and the *CYP2C* cluster to inform warfarin dosage [4], and GWAS made an important contribution to this [5]. For many other examples, the results to date are more equivocal.

A GWAS of response to TNF inhibitors in rheumatoid arthritis (RA) was recently conducted on behalf of Biologics in RA Genetics and Genomics Study Syndicate (BRAGGSS) and MAXimizing Therapeutic Utility in RA (MATURA), two UK consortia investigating response to treatment [6]. Previous studies had shown little consistency, and this study was larger, based on 1752 patients. No statistically significant associations were found overall, but there were some interesting associations when stratified by drug. We recently carried out a GWAS of response to methotrexate (MTX) in patients with RA [7], also on behalf of the MATURA consortium; this also came up with interesting but inconclusive findings. Involvement in these studies has led to the following personal reflections on the factors that make GWAS of therapeutic response more difficult than GWAS of disease or of a readily measurable trait.

Heritability

One issue that is peculiar to pharmacogenomics is that the usual procedure of first establishing and estimating heritability cannot readily be followed. For common diseases, GWAS can generally be conducted confident in the knowledge that there are genetic variants still to be discovered, since heritability has been estimated from family studies. This route is not usually possible in pharmacogenomics, and methods of estimating heritability using genome-wide data on unrelated individuals require very large sample sizes to provide reliable estimates (see below). Although there are known examples of genetic predictors of drug response, in most specific instances there is no good prior estimate of the genetic contribution to drug response. It is therefore possible that heritability of drug response in some contexts is extremely low, with almost all of the variability attributable to nongenetic features, such as the disease course and the concomitant environment, including comorbidities, other treatments and lifestyle.

Outcome measures

A second major issue is the definition of outcome. Unlike a binary disease state or a simple quantitative measure, such as height, therapeutic response to a drug can be measured in many ways. In the recent GWAS of response to MTX in RA [7], the primary outcome measure was change in disease-activity score (DAS28) from baseline to 6 months. Secondary analyses included components of DAS28 (swollen joint count, tender joint count and CRP). However, other ways of measuring outcome could have been used, and indeed have been used in other studies. These include categorical measures (e.g., nonresponder, poor responder and good responder) and longitudinal measures of outcome [8]. This raises important questions about how to select the most appropriate measures, and how to compare or combine results across studies.

If available, evidence of greater heritability of some measures compared with others could assist in making a more rational selection. Massey *et al.* [6] used a Bayesian approach to estimate the heritability of the different measures of response to TNF inhibitors; 6-month change in swollen joint count and in erythrocyte sedimentation rate (a marker of inflammation) were found to be more heritable than other measures considered, although the 90% credibility intervals were very wide (0.08–0.69 and 0.09–0.81, respectively).

Evidence of reliability or objectivity of measures could also be considered in choosing outcomes. In addition to greater estimated heritability, measures of inflammation (CRP) and swollen joint count are also the components of DAS28 that have shown more evidence of association with imaging-detected synovitis and radiographic progression [9,10].

It could alternatively be argued that the outcome measures should be based on clinical usage. The best choices in fact depend on context; for the discovery of associated genetic variants, a more direct, possibly intermediate or partial, measure of biological state is likely to be more suitable, whereas at the stage of biomarker evaluation for implementation a more composite clinically actionable measure may be necessary.

Different outcome measures in different studies make meta-analysis more complex than in a case–control setting, although an efficient meta-analysis can still be conducted on the basis of weighted Z scores, which take into account both the p-value and direction of effect [11].

Sample size

The main successes in discovering the polygenic basis of disease have come through collaborative GWAS bringing together sample sizes in the many thousands. Similarly, to increase sample size in pharmacogenomics, large international collaborations will be necessary, some of which already exist [3]. Differences in treatment protocols and health systems across countries will make this more challenging. Another way of achieving large sample sizes may be to use electronic health records, although this requires linkage to genetic data. Genomic data on over 16,000 participants in the Estonian Biobank have recently been used to carry out pharmacogenomic studies of adverse drug effects by linking genomic data to health records and prescription information, with some success [12]. Extending this approach to studies of therapeutic response may be difficult since outcome measures may not be well recorded in routine clinical practice.

Most ‘discovery’ pharmacogenomic studies consider a cohort of patients receiving one treatment and measure the association between genetic factors and outcome. To show that the outcome is due to a specific drug, and not just a measure of disease course which may not be treatment specific, it would be necessary to compare with a ‘control’ group not receiving the same treatment (gene by treatment interaction). Sample sizes adequate to this task, particularly if the genetic effect is weak or moderate, are not likely to be attainable at a genome-wide level, but could be achievable for specific associated variants.

Heterogeneity

Heterogeneity is the final major problem in pharmacogenomics. In the recent MTX GWAS [7], study entry criteria were applied to try to create a fairly homogeneous sample. Specifically, all subjects were of European ancestry and received MTX monotherapy as first-line treatment within 12 months of disease diagnosis. Disease activity was measured at baseline and (as far as possible) 6 months after the start of treatment. Nonetheless there remained considerable heterogeneity, for example in the MTX dosage regimen. In addition, when medication is self-administered, the actual dose may not be the same as the prescribed dose.

In any study there is likely to be a trade-off between homogeneity and sample size, and the best strategy may depend on the exact aims of the study. For ‘discovery’, it may be appropriate to increase sample size by combining information on response to the same drug across different diseases (e.g., RA and juvenile idiopathic arthritis [7]). In particular, genetic variants that affect drug metabolism may be expected to have a pan-disease effect. Conversely, if the aim is to advise on treatment options in a particular group of patients then the emphasis should be on homogeneity.

Future perspective

The stability and measurability of genetic variants make them very attractive as potential predictive biomarkers. In line with other GWAS, pharmacogenomic studies even of modest sample size would be capable of detecting variants of large effect. This seems reason enough to conduct them, even if usually no such variants are to be found.

The chance of studies of therapeutic response successfully identifying polygenic effects may be increased by careful consideration of the above issues of outcome measure, sample size and heterogeneity, with the best choice depending on the study aims. As the field progresses, larger sample sizes may be achievable through international collaboration and the wider availability of electronic health records, aided by greater consistency of outcome reporting. ‘Post-GWAS’ analyses [13] will need to be applied to fully exploit the results from these difficult-to-conduct studies, including pathway analyses and the incorporation of other -omics data.

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