How informative is a negative finding in a small pharmacogenetic study?

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Many pharmacogenetic studies fail to yield any statistically significant associations. Such negative findings may be due to the absence of, or inadequate statistical power to test for, an effect at the genetic variants tested. In many instances, sample sizes are small, making it unclear how to interpret the absence of statistically significant findings. We demonstrate that the amount of information that can be drawn from a negative study is improved by incorporating statistical power and the added context of well-validated pharmacogenetic effects into the interpretation process. This approach permits clearer inferences to be drawn from negative findings, such that no significant, the impact of sample size on the interpretation, and the dependence of power on minor allele frequency.

One way to address these shortcomings is to adopt a Bayesian approach, such as estimating the posterior probability of association. Other useful tools include confidence intervals for effect size and the careful investigation of power to determine what effect sizes could be detected from the study at hand and, thus, what effects sizes can be confidently excluded.

Additional inference can be drawn from negative studies by placing upon the graph points corresponding to well-known pharmacogenetic effect sizes associated with various medicines and clinical outcomes (Supplementary Tables 1 and 2) over the power curves for selected levels (see Statistical Methods in Supplementary Material). We can use power levels to differentiate the kind of effects we are likely to miss (e.g., power of 5%), have a reasonable chance of missing (50%) and are very unlikely to miss (95%). By adding the 95% simultaneous confidence intervals of effects estimated for each variant tested, we can assess the range of plausible effects given the observed data.

We demonstrate this power-based approach to interpretation using three examples selected from recent studies we have conducted. The first example is based on a pharmacogenetic study of pazopanib-related liver enzyme elevation, consisting of the analysis of 48 cases and 94 controls. For the given sample size, the effect sizes for almost all well-established adverse drug reactions lie above the 95% power curve (Figure 1a). Consequently, rejection would have been very likely if similar effects were present among the genetic variants tested. The second example illustrates our method for severe cutaneous adverse reactions in patients who received lamotrigine, consisting of 10 cases and 43 controls (Figure 1b). These power curves indicate that only the largest reported effects could be confidently ruled out. The third example is modeled after a pharmacokinetic

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study investigating drug exposure in 129 subjects (Figure 2). As in the first example, we can confidently exclude the presence of effect sizes observed for most large pharmacokinetic effects. We also applied the Bayesian posterior probability of association to these studies (data not shown). We did not find this measure to provide much additional insight beyond the confidence intervals.

The combination of power curves, observed effects and examples of well-known effects can help researchers to draw meaningful information about potential pharmacogenetic effects from otherwise ambiguous results. Most of this information is derived from the magnitude of the effects that are likely to be rejected by the study. As expected, the information in a negative study depends strongly on the sample size. For larger negative studies, we can exclude a large part of the effect size space, including most well-known effect sizes with demonstrated clinical utility. Such negative studies are informative, because they confidently exclude the existence of large effects at the measured variants. On the other hand, for a small study, we might have sufficient power to exclude only the largest possible effect sizes, and little power to exclude most known effects. Consequently, the information gained from such a negative finding is low, and researchers should be cautious drawing any conclusions.

Figure 1 Power at a type I error of $5 \times 10^{-4}$ (simultaneous testing of 100 variants) for pazopanib (a) and lamotrigine (b) studies investigating whether selected human leukocyte antigen genotypes are associated with adverse drug reactions. Data-derived features presented in the plot are the estimated odds ratios (OR; blue horizontal segments) for individual variants, their 95% simultaneous confidence intervals (green) and red power curves corresponding to 95% (solid), 50% (dashed) and 5% (dash-dotted) power. ORs for drugs with well-known pharmacogenetic effects are plotted as magenta star characters with the following abbreviations: Aba, Abacavir; Aug, Augmentin; All, Allopurinol; Car, Carbamazepine; Flu, Flucloxacillin; Iri, Irinotecan; Iso, Isoniazid; Lap, Lapatinib; Lum, Lumiracoxib; Mer, Mercaptopurine; Tic, Ticlopidine; Tra, Tranilast.

Figure 2 Power at a type I error of $5 \times 10^{-4}$ for a pharmacogenetic investigation of pharmacokinetic variation. Effect size measure is standardized mean difference, described in Online Methods. Drug abbreviations are as follows: Ato, Atomoxetine; Clo, Clopidogrel; Des, Desipramine; Mer, Mercaptopurine; Ome, Omeprazole; Phe, Phenytoin; War, Warfarin. See the legend to Figure 1 for further details.
Although for illustration purposes we used the power curves method retroactively, this method would provide maximal benefit when used in the experiment design stage. At this stage, the power curves can be produced to inform the researchers about the kinds of effects that could be detected and rejected in a proposed study. On the basis of estimated power for well-known effects, scientists will be better able to predict the possible consequences of the proposed genetic study.

Conflict of interest

The authors declare no conflict of interest.

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