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A Computational Approach to Identify Interfering Medications on Urine Drug Screening Assays without Data from Confirmatory Testing

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Abstract

Urine drug screening (UDS) assays can rapidly and sensitively detect drugs of abuse but can also produce spurious results due to interfering substances. We previously developed an approach to identify interfering medications using electronic health record (EHR) data, but the approach was limited to UDS assays for which presumptive positives were confirmed using more specific methods. Here we adapted the approach to search for medications that cause false positives on UDS assays lacking confirmation data. From our institution's EHR data, we used our previous dataset of 698,651 UDS and confirmation results. We also collected 211,108 UDS results for acetaminophen, ethanol and salicylates. Both datasets included individuals' prior medication exposures. We hypothesized that the odds of a presumptive positive would increase following exposure to an interfering medication independently of exposure to the assay's target drug(s). For a given assay–medication pair, we quantified potential interference as an odds ratio from logistic regression. We evaluated interference of selected compounds in spiking experiments. Compared to the approach requiring confirmation data, our adapted approach showed only modestly diminished ability to detect interfering medications. Applying our approach to the new data, we discovered and validated multiple compounds that can cause presumptive positives on the UDS assay for acetaminophen. Our approach can reveal interfering medications using EHR data from institutions at which UDS results are not routinely confirmed.

Introduction

Urine drug screening (UDS) assays play a role in various clinical contexts, from the emergency department to outpatient rehabilitation and treatment centers. Because UDS assays prioritize sensitivity over specificity, positive UDS results can occur due to interference by non-targeted substances, such as other medications (1). For this reason, positive UDS results are considered presumptive until confirmed by a more specific technique such as mass spectrometry. Results of confirmatory testing, however, are often not available until several days later, and many hospitals do not confirm presumptive positives at all. Better knowledge of the substances that cause false positives would help laboratorians and physicians who rely on UDS results to guide patient care.

Recently, we developed and validated an approach, based on statistical analysis of electronic health record (EHR) data, to identify interfering medications that cause false positive UDS results (2).

In this initial work, we relied on confirmation data to determine whether each presumptive positive was a true positive or a false positive. For hospitals where presumptive positives are not automatically confirmed, however, our approach is not applicable.

In this study, we extended our approach to identify medications capable of causing false positives on UDS assays that lack confirmation data. We validated the new approach by comparing it against our original approach on a dataset of nearly 700,000 paired UDS-confirmation results for 10 classes of target drugs. As proof of principle, we then applied the new approach to data from three UDS assays for which our institution does not automatically confirm presumptive positives.

Methods

The Vanderbilt Institutional Review Board reviewed and approved this study as non-human subjects research (IRB# 081418 and 190165).

Extraction of UDS results and drug exposures from EHR data

EHR data came from the Synthetic Derivative, a collection of deidentified clinical data from Vanderbilt University Medical Center (VUMC) [3]. In addition to using the dataset from our previous study [2], we made a new dataset consisting of all UDS results for the currently used assays for acetaminophen, ethanol and salicylates. The acetaminophen (Sekure Chemistry REF L3K Rev 9/17, Burlington, MA) and salicylates (Abbott Multigent REF 3K01-20 Rev 3/17, Lake Bluff, IL) assays were validated as laboratory-developed tests, as they are not FDA-cleared for use in urine. No other changes were made to the assay parameters. The validations were performed in accordance with the College of American Pathologists' criteria and are in routine clinical use. The ethanol assay (Abbott Multigent REF 3L36-20 Rev 2/17) was FDA-cleared for use in urine. All testing was performed on an Abbott Architect c16000 automated chemistry analyzer. All three assays are validated to produce quantitative results, which are converted clinically to presumptive positive or negative results based on laboratory-defined cutoffs (Table I). The Synthetic Derivative contains only the qualitative results.

For each person in the new dataset, we identified drug exposures documented between 1 and 30 days prior to each UDS result. We excluded UDS results that occurred less than 30 days after the person's first ever visit at VUMC, since we would lack a prior 30 days of documented drug exposures. Documented drug exposures are available as structured data in the Synthetic Derivative and come primarily from medication lists. We mapped each drug to its active ingredient(s), which include prodrugs, using RxNorm [4]. For simplicity, we refer to these active ingredients as medications in the rest of the manuscript.

As described previously, having a documented exposure within 30 days is only a proxy for being exposed at the time of providing the urine sample [2]. For example, even if a person is taking a medication every day, the medication list is only updated when the person visits a healthcare provider. Thus, the proxy is valid even if the medication's half-life is less than 30 days. As this is a retrospective analysis from EHR data, it is impossible to verify the presence of every medication in every patient sample.

Statistical analysis of drug exposures and UDS results

We quantified associations between drug exposures and UDS results using Firth's logistic regression [5, 6]. Given the coefficients and standard errors from the logistic regression fits (where each coefficient corresponded to a log odds ratio), we then used an Empirical Bayes approach called adaptive shrinkage to estimate the posterior mean of the log odds ratio and the corresponding 95% credible interval for each assay–medication pair [7]. The latter is analogous to a confidence interval, but for Bayesian statistics.

For the re-analysis of our previous dataset, we fit two types of logistic regression models. In model 1, the dependent variable corresponded to the UDS result (negative or false positive) and the independent variable corresponded to presence or absence of prior exposure to the medication. In model 2, the dependent variable corresponded to the UDS result (negative or presumptive positive) and the independent variables corresponded to (i) presence or absence of prior exposure to the medication and (ii) presence or absence of prior exposure to the assay's target drug(s) (if not the same as the medication of interest). For consistency with our previous study, we only fit a model for an assay–medication pair if exposure to the medication preceded a false positive (model 1) or presumptive positive (model 2) in at least five individuals.

For the analysis of our new dataset, we fit model 2 for assay–medication pairs for which exposure to the medication preceded a UDS result in at least 20 individuals. Given the lack of confirmation data for these assays, we designed this cutoff to exclude extremely rare medications and to potentially identify medications associated with both higher and lower rates of presumptive positives. For the medications most strongly associated with presumptive positive results on the acetaminophen assay, we calculated co-exposure frequencies as the percentage of exposures to one medication for which the person was also exposed to a second medication.

Experimental validation of interference

For each selected compound, we spiked a reference standard into drug-free urine at various concentrations and tested the spiked urine samples in singlicate on an Abbott Architect c16000 chemistry analyzer. Because all three assays are validated quantitatively, we used the numeric result rather than the qualitative interpretation. We then used linear interpolation to estimate the concentration of the test compound at which the assay registered a concentration equal to the cutoff.

We purchased reference standards from Tocris Bioscience (Bristol, UK). We prepared stock solutions of each standard

Table I. Characteristics of Newly Analyzed Urine Drug Screening Assays

Target drug	Format	Manufacturer/brand	Cutoff (μg/mL)	Number of UDS results	
				Negative	Presumptive positive
Acetaminophen	Enzymatic (acyl amidohydrolase)/colorimetric	Sekure Chemistry/L3K Assay	3	54,220	16,180
Ethanol	Enzymatic (alcohol dehydrogenase)/colorimetric	Abbott MULTIGENT	100	65,012	5,473
Salicylates	Enzymatic (salicylate hydroxylase)/colorimetric	Abbott MULTIGENT	5000	62,692	7,531

in DMSO (carbidopa and entacapone), or in saline and HCl (levodopa). We spiked the urine samples using a fixed volume of 20% spiking solution, made of a combination of diluent and stock solution. Stock solutions made in DMSO were diluted in saline/DMSO mixtures to ensure that the organic content did not vary with drug concentration. To ensure that the diluents did not cause false positive results, negative controls were prepared with matching proportions of saline and/or DMSO and tested with each batch of samples. In most cases, we tested up to the maximum technically feasible concentration for a compound, given the limits of solubility, the concentration of the reference material, and the fixed 20% spiking volume.

Results

Without the confirmation results, one cannot know whether a given presumptive positive UDS result was a true positive or a false positive. We hypothesized, however, that the odds of a presumptive positive would increase following exposure to an interfering medication independently of exposure to the assay's target drug(s). To test this hypothesis, we used the dataset from our previous study [2], which included results from urine drug screens and confirmations for 10 classes of target drugs, as well as each person's prior documented medication exposures (Supplementary Table 1).

We used logistic regression followed by a technique called adaptive shrinkage [7] to quantify two types of associations: (i) between medication exposures and false positive UDS results, yielding an odds ratio OR_{FP}, and (ii) between medication exposures and presumptive positive UDS results, adjusted for exposure to assay targets and yielding an odds ratio OR_P (Supplementary Tables 2 and 3). Thus, whereas OR_{FP} used the confirmation results as in our previous approach, OR_P did not.

We compared OR_{FP} and OR_P for assay targets, previously known interferents, and 'new' interferents that we discovered in our previous study (Figure 1). Consistent with our hypothesis, most interfering medications with a high OR_{FP} also had a high OR_P (Figures 1A and B). In addition, ranking by OR_P captured only moderately fewer interfering medications than ranking by OR_{FP} (Figure 1C). Thus, our approach can detect medications that may cause false positive UDS results, even if confirmation data are unavailable.

To apply our approach to new data, we extracted all UDS results for acetaminophen, ethanol and salicylates, three assays for which presumptive positive results at our institution are not confirmed. The dataset included 211,108 results from 39,658 individuals (Table I), along with each person's documented drug exposures occurring between 1 and 30 days prior.

Using the new dataset, we calculated OR_P for 2,563 assay–medication pairs. Further supporting our approach's validity, acetaminophen was the fourth-ranked medication on the acetaminophen assay and aspirin was the top-ranked medication on the salicylates assay (Figure 2). Ethanol as a medication had only 16 exposures in our dataset and showed no clear association with results on the ethanol assay (Supplementary Table 4).

The three medications that had a higher OR_P than acetaminophen on the acetaminophen assay were levodopa, carbidopa and entacapone (Figure 2). These associations were unlikely to be due solely to co-exposure with acetaminophen, as each logistic regression model already accounted for exposure to the respective assay's target drug. Co-exposure analysis indicated that the associations of levodopa and carbidopa were indistinguishable because the two medications were almost always given together (Supplementary Figure 1). Subsequent logistic regression also suggested that entacapone's association with presumptive positive UDS results could be explained by co-exposure with levodopa and/or carbidopa (Supplementary Table 5).

We evaluated the interference of each of these three compounds experimentally (Figure 3). Consistent with our analysis of the EHR data, both levodopa and carbidopa interfered strongly on the acetaminophen assay, each producing a presumptive positive (corresponding to an acetaminophen concentration of 3 μg/mL) at less than 40 μg/mL. Entacapone, on the other hand, produced a presumptive positive at 400 μg/mL.

Levodopa and carbidopa are structurally related to alpha-methyldopamine, a metabolite of methyldopa that interferes on our institution's UDS assay for amphetamines [2]. Methyldopa was modestly associated with presumptive positive UDS results for acetaminophen ($OR_P = 1.32$, rank 44 of 854, Supplementary Table 4), and both methyldopa and alpha-methyldopamine—but not a second metabolite 3-o-methyldopa—were strongly interfering (Figure 3). Conversely, neither levodopa nor carbidopa interfered on the amphetamines assay (Supplementary Table 6). Taken together, these findings indicate that several dopamine-related compounds can cause false positive UDS results for acetaminophen.

Discussion

Despite UDS assays' vulnerability to interference, confirmatory testing is not always available, either for patient care or secondary analysis. Here we extended our previous approach in order to identify potentially interfering medications from EHR data without knowing which presumptive positive UDS results were true positives and which were false positives. The simple idea is to find medications associated with presumptive positives even after accounting for the screen's target drug(s).

We are fortunate at our institution that presumptive positive results for most UDS assays, unless ordered through the emergency department, are routinely confirmed. This allowed us to rigorously compare, in terms of ability to detect interfering medications, the approach that does not require confirmation data to the approach that does. Although the clinical utility of the UDS assays for acetaminophen, ethanol and salicylates is generally low, we used data from these assays as proof of principle that our approach could successfully identify interfering medications without requiring confirmation data.

EHR data are noisy, and the reliability of a medication's odds ratio depends on the accuracy with which exposures to that medication are documented in the EHR. More importantly, EHR data are observational, which means our approach only quantifies correlations. It does not attempt to explain which medication(s) may have caused a given presumptive positive result. Thus, the decision of which compounds to experimentally evaluate for interference should involve both the statistical analysis and clinical expertise. For example, we did not pursue the top-ranked medications on the ethanol assay (thiamine, diazepam and naltrexone) because we considered it likely that the associations were due to confounding with alcohol-use disorder. In the future, accounting for such underlying patient phenotypes could further improve our approach.

Furthermore, within our dataset, we cannot know what caused the presumptive positive acetaminophen screens in individuals exposed to carbidopa and levodopa. In general, determining whether a given drug caused a given lab result is extremely challenging and can involve dosing human volunteers and performing repeated
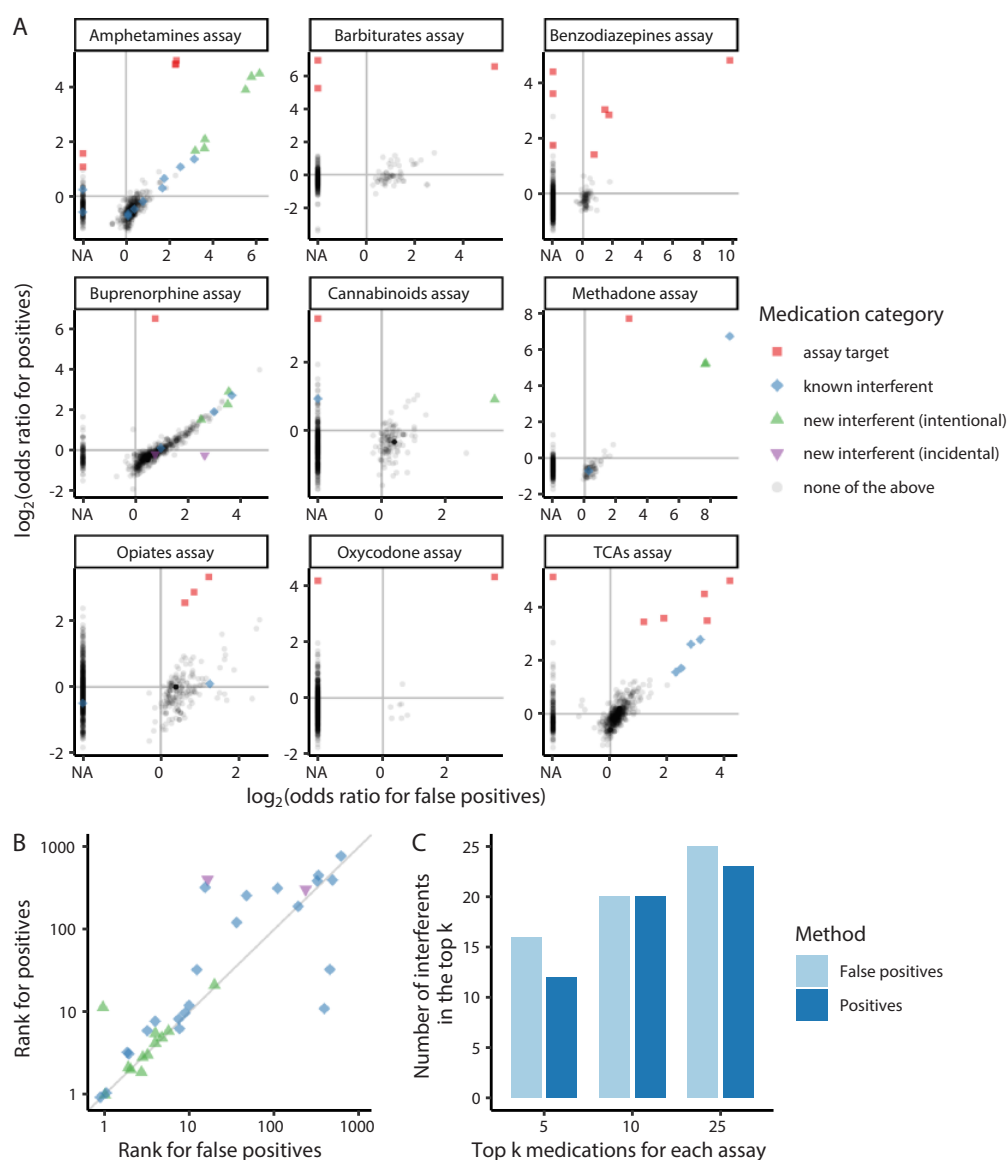
Figure 1. Comparison of calculating potential interference with and without confirmation data. (A) Each plot corresponds to a UDS assay, and each point corresponds to a medication. A log₂ (odds ratio for false positives) of NA indicates that the association was not tested because less than five individuals had a false positive UDS result preceded by exposure to the given medication. The assay for cocaine metabolite is not shown as it had no false positives. New interferent (intentional) indicates a medication we validated in our previous study based on its high odds ratios for false positives on the given assay. New interferent (incidental) indicates a medication we validated on a different assay than the one for which it had a high odds ratio. (B) Ranks of interfering medications on all assays based on odds ratio for false positives and odds ratio for presumptive positives. Ranks were calculated on a per-assay basis and excluded assay targets. Points are slightly jittered to avoid overlap. (C) Number of interfering medications across all assays with an odds ratio in the top k for each assay (where $k = 5$, 10 or 25), excluding assay targets.

testing. Our findings do indicate, however, that (i) individuals with a documented exposure to carbidopa and levodopa are more likely to screen positive and (ii) carbidopa and levodopa can cause positive screens on their own. This combination of empirical association and experimental validation provides evidence that exposure to the medications is causal for some fraction of presumptive positive results.

Interestingly, the acetaminophen assay's package insert includes methyldopa and levodopa (but not carbidopa) on its list of potentially interfering substances but mentions neither the concentration at which interference was observed nor the type of interference (positive or negative). While one might assume that anything listed in the package insert will interfere, none of the other 14 listed compounds were in the top 100 in our analysis. In our experience, this combination of incompleteness and ambiguity typifies interference testing summaries of assay package inserts and highlights the need for more thorough and transparent reporting of assay interferences.

Our findings suggest that statistical analysis of EHR data could enable 'postmarketing surveillance' of an assay's performance in routine use. Given the limited number of assay manufacturers,
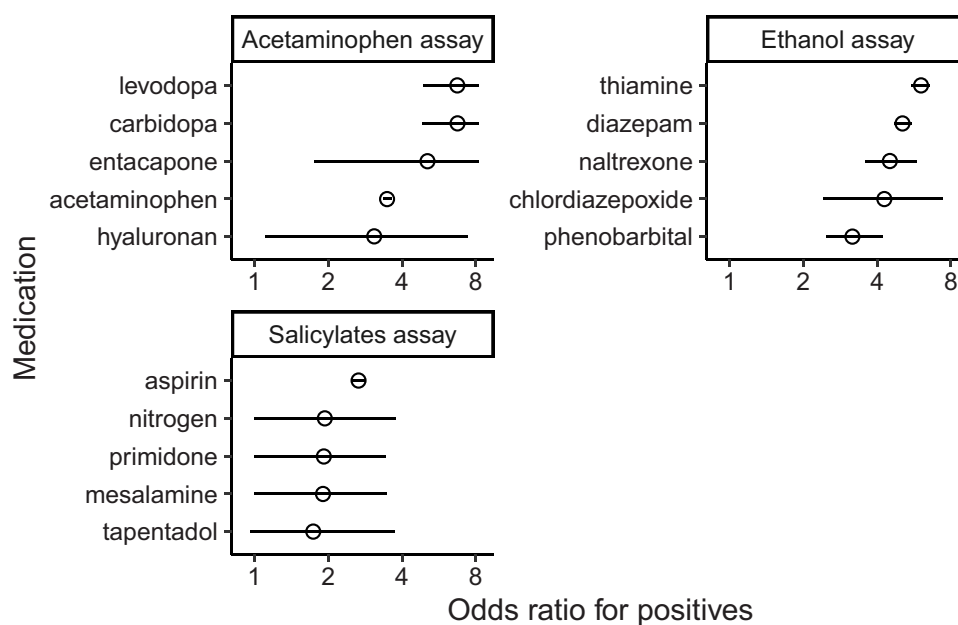
Figure 2. Top-ranked medications associated with presumptive positive UDS results for the acetaminophen, ethanol and salicylates assays. Error bars indicate 95% credible intervals. All tested associations for the three assays are in Supplementary Table 4.
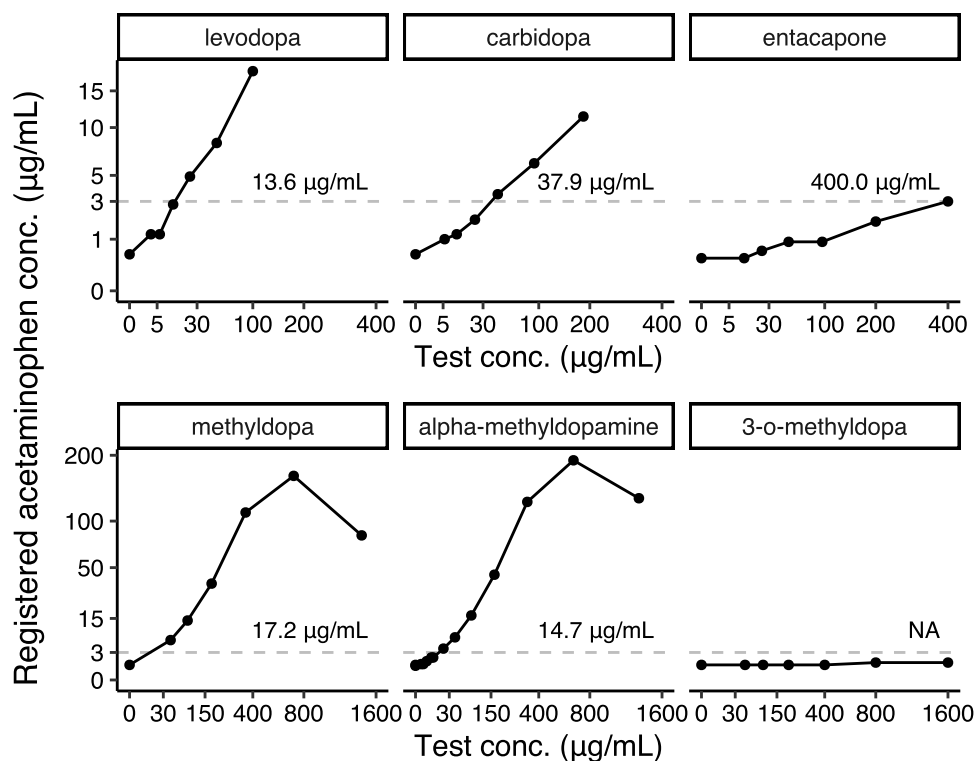
Figure 3. Experimental validation of interference on the acetaminophen assay. Dashed lines show the cutoff normally used to call a sample presumptive positive. The text above each dashed line indicates the estimated minimum concentration at which the test compound would produce a presumptive positive. Both axes are square-root-transformed to better show the lower concentrations.

implementation of surveillance programs at several large sites could be sufficient to uncover the majority of interfering medications. This information could then be disseminated to benefit the entire laboratory community.

Funding

Data availability

Code and summary-level data for this study are available at https://doi.org/10.6084/m9.figshare.12067233.

Supplementary data

Supplementary data is available at *ANATOX Journal* online.

References

1. Saitman, A., Park, H.-D., Fitzgerald, R.L. (2014) False-positive interferences of common urine drug screen immunoassays: a review. *Journal of Analytical Toxicology*, **38**, 387–396. 10.1093/jat/bku075
2. Hughey, J.J., Colby, J.M. (2019) Discovering cross-reactivity in urine drug screening immunoassays through large-scale analysis of electronic health records. *Clinical Chemistry*, **65**, 1522–1531. 10.1373/clinchem.2019.305409
3. Danciu, I., Cowan, J.D., Basford, M., Wang, X., Saip, A., Osgood, S., *et al.* (2014) Secondary use of clinical data: the Vanderbilt approach. *Journal of Biomedical Informatics*, **52**, 28–35. 10.1016/j.jbi.2014.02.003
4. Nelson, S.J., Zeng, K., Kilbourne, J., Powell, T., Moore, R. (2011) Normalized names for clinical drugs: RxNorm at 6 years. *Journal of the American Medical Informatics Association: JAMIA*, **18**, 441–448. 10.1136/amiajnl-2011-000116
5. Bias, F.D. (1993) Reduction of maximum likelihood estimates. *Biometrika*, **80**, 27–38. 10.1093/biomet/80.1.27
6. Heinze, G., Schemper, M. (2002) A solution to the problem of separation in logistic regression. *Statistics in Medicine*, **21**, 2409–2419. 10.1002/sim.1047
7. Stephens, M. (2017) False discovery rates: a new deal. *Biostatistics*, **18**, 275–294.