Combined analysis of phase I and phase II data to enhance the power of pharmacogenetic tests

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\textbf{ABSTRACT}

We show through a simulation study how the joint analysis of data from phase I and phase II studies enhances the power of pharmacogenetic tests in pharmacokinetic (PK) studies. PK profiles were simulated under different designs along with 176 genetic markers. The null scenarios assumed no genetic effect, while under the alternative scenarios, drug clearance was associated to 6 genetic markers randomly sampled in each simulated dataset. We compared penalised regression Lasso and stepwise procedures to detect the associations between empirical Bayes estimates of clearance, estimated by nonlinear mixed effects models, and genetic variants. Combining data from phase I and phase II studies, even sparse, increases the power to identify the associations between genetics and PK due to the larger sample size. Design optimisation brings a further improvement, and we highlight a direct relationship between $\eta$-shrinkage and loss of genetic signal.

\textbf{KEYWORDS}

Pharmacogenetics, Pharmacokinetics, Nonlinear mixed effects models, Design, Clinical drug development.
ABBREVIATIONS

1. EBE: Empirical Bayes estimates
2. FWER: Family wise error rate
3. FIM: Fisher information matrix
4. GAM: Generalised additive model
5. $H_0$: Null scenarios
6. $H_1$: Alternative scenarios
7. IRIS: Institut de Recherches Servier
8. IIV: Interindividual variability
9. LD: Linkage disequilibrium
10. LRT: Likelihood ratio test
11. NCA: Noncompartmental analyses
12. NLMEM: Nonlinear mixed effects models
13. PK: Pharmacokinetics
14. RD: Relative deviation
15. REE: Relative estimation error
16. $R_{GC}$: Genetic component of the interindividual variability
17. SE: Standard error
18. Shr: $\eta$-shrinkage
19. SNPs: Single nucleotide polymorphisms
20. TP: True positive
21. TPR: True positives rate
22. FP: False positive
23. FPR: False positives rate
24. $\alpha$: Type I error per test
25. $\beta$: Effect size coefficient
26. $\xi$: Tuning parameter for Lasso method
27. $\rho$: Correlation coefficient for stepwise procedure
28. $N_t$: Number of tests
29. $p_k$: Frequency of the minor allele
INTRODUCTION

Studying the sources of the variability observed in drug response facilitates individualisation of prescription. One of the sources of variability in drugs’ pharmacokinetics (PK) is the variation in activity of enzymes and transporters involved in the drug absorption, distribution, metabolism or elimination. Pharmacogenetics studies the genetic component of interindividual variability (IIV) observed in PK to identify populations at risk of treatment inefficacy or adverse effects. Single nucleotide polymorphisms (SNPs) are the genetic variants most frequently studied in pharmacogenetics and screened more and more often in clinical studies.

Genetic data offer some unique challenges, in particular because they may lead to very unbalanced number of subjects, which impacts the power of tests in pharmacogenetic analyses. In a previous simulation work, we showed that typical phase I studies have low power to detect genetic effects because of the limited sample size. On the other hand, phase I studies generally provide good quality PK information allowing to characterise the PK profile of the drug. We showed that from the different approaches used at this stage to estimate PK parameters, nonlinear mixed effects models (NLMEM) could be considerably more powerful than noncompartmental analyses (NCA) for complex PK models. Our simulations also showed that increasing the sample size, as in phase II studies, would improve the power to detect genetic variants. However sparse designs typically used in phase II may result in biased estimations for empirical Bayes estimates (EBE) used in GAM covariate analysis procedure (generalised additive models).

To increase the detection of genetic covariates, a way could be to combine for the analysis data from a study collected with a rich design, as expected in phase I, with sparser, but still informative, data from a phase II study.

In the present work we propose practical designs involving phase I and phase II data, and we quantify through simulations their ability to detect genetic associations with PK. A motivating example was provided by IRIS, a pharmaceutical industry, to generate realistic genetic and PK data. We compared two association methods, a penalised regression method and a stepwise procedure.
MATERIALS AND METHODS

Simulation study

Figure 1 presents the framework of the simulation study, which was designed based on PK data from drug S (IRIS) collected in 78 subjects from 3 phase I clinical studies\(^{11}\). All subjects were genotyped at baseline using a DNA microarray developed by IRIS of 176 SNPs known for being involved in the PK of drugs. These 176 polymorphisms were matched to a reference Hapmap panel (Hapmap 3 release 2) for a Caucasian population\(^{12}\) and we used the Hapgen2 software\(^{13}\) to simulate genetic variants retaining their frequencies and the correlations between polymorphisms found in the human genome (see details in Supplementary Material, Supplementary Figures S1-S3).

PK profiles were simulated with a two-compartment model with dose-dependent double absorption (Supplementary Figure S4), with the parameters in Table 1, under two conditions: (i) no gene effect (H\(_0\)); (ii) gene effect on clearance CL (H\(_1\)). Under H\(_1\), 6 SNPs were drawn randomly without physiological assumptions or prior knowledge, and assumed to explain in total 30% of the IIV on CL through the following additive genetic model on the log-transformed CL:

\[
\log(CL_{sim_i}) = \log(\mu_{CL}) + \sum_{k=1}^{6} \beta_k \times SNP_{tk} + \eta_{i,CL}\\
\]

where \(CL_{sim_i}\) is the simulated individual clearance, \(\mu_{CL}\) the typical clearance, \(\beta_k\) the effect size associated to the variant allele of \(SNP_{tk}\) and \(\eta_{i,CL}\) the interindividual random effect for clearance of subject \(i\). Causal SNPs were different from one dataset to another. Assuming an additive genetic model, genotypes take values 0, 1 or 2, reflecting the number of mutated alleles. We chose this model to simplify the simulations but dominant or recessive genetic models could be easily simulated by changing genotype values. \(\beta_k\) was computed as a function of the coefficient of genetic component (\(R_{GCC,k}\), the percentage of the interindividual variability in CL explained by the SNP) and the minor allele fraction \((p_k)\), as follows:

\[
\beta_k = \frac{R_{GCC,k} \times \omega^2_{CL}}{\sqrt{2p_k(1-p_k) - R_{GCC,k} \times 2p_k(1-p_k)}}\\
\]

where \(\omega^2_{CL}\) is the variance of interindividual random effects on CL due to non-genetic sources. \(R_{GCC,k}\) was respectively equal to 1, 2, 3, 5, 7 and 12% for the 6 causal variants\(^{14}\) to
mimick a multifactorial genetic effect. Then under $H_0$, $\omega^2_{CL} = 0.06$ (as in Table I), while under $H_1$ 30% of the variance is explained by the genetics so that $\omega^2_{CL} = 0.04$ (example on the magnitude of simulated effect sizes is available in Supplementary Table S1). The simulated datasets were then fitted with the base model without genetic covariates. Individual clearance estimates ($E_B E_{CL_i}$) were estimated and all associations with the 176 simulated polymorphisms were tested assuming a linear relation without re-estimating model parameters, as in a GAM analysis. We compared two association methods to detect gene effects. Lasso is a multivariate penalised regression which simultaneously estimates effect size coefficients and selects variants by setting a large number of coefficients to 0. The penalty is set by a tuning parameter ($\xi$) which depends on $\alpha$, the type I error per test, and the number of subjects ($n$) (Figure 1). Alternatively in practice the penalty can be determined through permutation or cross-validation methods, which are more time consuming. Stepwise procedure includes relationships one by one depending on the significance of a Wald test compared to a threshold $\alpha$. The correlation between two significant SNPs, due to linkage disequilibrium, is computed through the Pearson’s correlation coefficient $r$ and if two significant SNPs are strongly correlated ($|r| > 0.89$), only the most significant is kept. Finally the most significant variant among selected SNPs is kept in the final model and steps are repeated until no more association is significant (Figure 1).

In both approaches, we control the Family Wise Error Rate (FWER), representing the percentage of datasets where at least one variant is selected under $H_0$, by correcting the nominal $\alpha$ by the number of tests performed (Sidak correction) corresponding to the number of polymorphic SNPs considered $N_t$ (Figure 1). The FWER was set to 20% (with a prediction interval for 200 datasets equal to [14.5-25.5]) for an exploratory analysis. The prediction interval determined when to adjust $\alpha$ to control the FWER under $H_0$.

Simulated designs and analysis scenarios

We simulated a phase I study corresponding to the motivating example, including 78 subjects (N1) receiving 8 different single doses (5, 10, 20, 50, 100, 200, 400 or 800 units, for respectively 6, 6, 24, 12, 12, 6, 6 and 6 subjects per dose) and sampled at 16 times. Three designs of phase II study were simulated. They included 306 subjects (N2), receiving 3 doses (20, 50 or 100 units, 102 subjects per dose), sampled at steady state. Two phase II studies
included three samples per subject, optimised using the PFIM software\textsuperscript{18} to ensure a reasonable precision of CL estimates. The last sampling time was limited to 24h in one, while a late sample was allowed after the last dose administration in the other. The third study included only one trough concentration (24h). We considered 4 analysis scenarios (Figure 1), three combining the phase I and one of the phase II study (respectively SPI/II\textsubscript{3s.24h}, SPI/II\textsubscript{3s.96h} and SPI/II\textsubscript{1s.24h}), and one, for comparison, with only the phase I subjects (SPI).

We also investigated the impact of a higher variability on phenotype on the results. For this, we simulated the same four scenarios increasing the IIIV on CL to 60\% (instead of 25\% in previous settings).

**Evaluation**

For each analysis scenario, 200 datasets were simulated under $H_0$ and $H_1$. The ability of the designs to estimate the population and individual parameters under $H_0$ was first evaluated through estimation bias and $\eta$-shrinkage (see details in Supplementary Material, Supplementary Figures S5-S6).

Under $H_1$ we evaluated the performance of each scenario in terms of true and false positives counts (TP and FP) and rates (TPR, the proportion of TP detected among the causal variants; and FPR, the proportion of FP detected among all potential false associations) for parameter CL, as well as the probability to detect genetic variants. Assuming that SNPs located on genes coded for metabolism enzymes and transporters affect mostly the drug distribution and elimination, we also applied association tests on Q, the intercompartmental clearance and V2, the peripheral volume, separately. Any variants associated to Q and V2 were counted as false positives. The central volume V1 was not considered because it had no random effects.

We also evaluated the loss of genetic signal between simulated and estimated individual clearances, comparing slopes $b$ of univariate linear regressions on $\log(\text{CL}_{\text{sim}_i})$ or $\log(\text{EBE}_{\text{CL}_i})$ for each causal variant. A relative deviation of the genetic signal $RD_{\text{signal}}$ was computed as follows:

$$RD_{\text{signal}}(\%) = \frac{b_{\text{EBE}_{\text{CL}_i}} - b_{\text{CL}_{\text{sim}_i}}}{b_{\text{CL}_{\text{sim}_i}}} \times 100$$

(3)

$RD_{\text{signal}}$ quantifies the departure of the estimated genetic signal ($b_{\text{EBE}_{\text{CL}_i}}$) from the one simulated ($b_{\text{CL}_{\text{sim}_i}}$, see details in Supplementary Material).
RESULTS

Control of FWER under $H_0$

Lasso and stepwise procedure both tended to be too conservative, as the FWER was lower than expected in some scenarios (Table 2). After an empirical correction by increasing the type I error per SNP $\alpha$, FWER was properly controlled around 20%. This correction was applied in the corresponding simulation under $H1$. Previous simulations suggested that this decrease in FWER is influenced by correlations between polymorphisms\textsuperscript{6}.

Detection of genetic effects

Under $H_1$ the TPR (Figure 2, top left) was higher in scenarios including phase II data (from 22 to 32%) compared to scenario with only phase I data (SPI, 4%) and was the highest in scenario SPI/I\textsubscript{I35.96h}. The FPR was lowest (0.2%) in scenario SPI where a limited number of SNPs was selected, and only slightly higher in scenarios including phase II data, ranging from 0.6 to 0.8% for both methods. Very few TP were effectively detected in scenario SPI (around 44 for both methods) where the number of subjects was limited (N1 = 78) (Supplementary Tables S2-S3). By adding more subjects (N2 = 306) to the analysis, the number of TP increased sharply. Scenario SPI/I\textsubscript{I35.96h} allowed detecting the largest number of TP (380 or more), while in SPI/I\textsubscript{I35.96h}, around 326 TP were detected. In SPI/I\textsubscript{I15.24h} the number of TP was lower (around 270 TP), but remained much higher than scenario SPI with only phase I data. In the same way, the number of FP increased when including phase II data to the analysis, but to a much lesser extent.

With only phase I data, the probability to detect at least one genetic variant on CL was low (Figure 2, bottom left), around 20% (SPI). This probability decreased quickly when trying to detect more polymorphisms and reached 0 for 3 variants or more. Adding phase II data to the analysis increased the probability to detect at least one variant about 85% in scenario SPI/I\textsubscript{I15.24h}, and up to 95% in scenario SPI/I\textsubscript{I35.96h}. Scenarios including phase II data showed good detection of 1 to 3 SNPs and SPI/I\textsubscript{I35.96h} had always the higher detection. This shows that the major determinant of power is the number of subjects, and that optimising the design for more informativeness can bring a smaller further improvement. The low probability to detect 4 SNPs or more ($\leq$ 4%) in scenarios combining phase I and phase II data can be explained by those variants having a very weak impact; polymorphisms only explaining 1, 2 or 3% of the variability of CL.
In Supplementary Table S4, the TPR was computed separately for each causal SNP. The variants associated to the lowest $R_{GC}$ had low TPR, close to the FPR. Thus the signal associated to these variants was close to the noise created by the non-causal variants.

**Shrinkage**

Two $\eta$-shrinkage estimates were computed using metric proposed by Bertrand et al.\textsuperscript{4} based on estimated variances, with respect to the estimate of $\hat{\omega}^2$ in the dataset; one over the $\hat{\eta}_i$ from phase I subjects and one over the $\hat{\eta}_i$ from phase II subjects (Figure 3). The $\eta$-shrinkage for phase I subjects was low (median = 23%) thanks to the large number of observations per subject. A large range of $\eta$-shrinkage estimates for phase II data was observed across analysis scenarios, but was below 50% in scenario SPI/II\textsubscript{3s.96h}.

**Loss of signal**

$RD_{signal}$ was always negative for the 6 SNPs, indicating that part of the signal was lost during the estimation step (Figure 4, top). This loss was smaller in the scenario with phase I data alone (SPI) than in scenarios combining phase I and phase II data. In each scenario the signal loss was of the same magnitude for the 6 SNPs, regardless of the value of associated $R_{GC}$. For phase I data (Figure 4, bottom), the loss was of a constant magnitude across scenarios (median = -30%). For phase II data, in the most informative scenario (SPI/II\textsubscript{3s.96h}) the loss was of a similar magnitude (median = -41%) than the loss in phase I data, where subjects were extensively sampled. The loss was higher in scenario SPI/II\textsubscript{3s.24h} (median = -56%), and even more when only one time was sampled (SPI/II\textsubscript{1s.24h}, median = -70%). The signal loss and $\eta$-shrinkage values changed accordingly across phase II scenarios, while the probability of detection changed in the opposite direction.

**Influence of the phenotype variance**

Increasing IIV for the CL parameter to 60% led to a sharp increase in the number of TP (Supplementary Tables S5-S6), resulting in higher TPR and higher probabilities to detect the causal variants (Figure 2, right), compared to when individual CL were simulated with a moderate IIV. This higher number of TP is explained first and foremost by the increase in simulated effect sizes which depended on the variance of interindividual random effects on CL due to non-genetic sources (equation 2). A second consequence of the larger IIV was that the estimated $\eta$-shrinkages became much smaller. Lower $\eta$-shrinkages resulted in lower
signal losses in all scenarios for phase I and phase II data (Supplementary Figure S7-S8), which again favoured a higher probability to detect the genetic effects.

**DISCUSSION**

In this work, we show and evaluate practical designs to combine data from studies occurring in phase I and II of a drug development. We assess through a simulation study, inspired by a real example, the probability to detect genetic variants and the influence of the phase II study design. We considered phenotypes estimated by NLMEM, which can handle the analysis of heterogeneous data involving sparsely sampled subjects. Genetic variants are unbalanced and so the amount of information they provide is directly related to the variant allele frequency and the study sample size. On the other hand, PK information depends also on the number and times of sampling which drives the precision of the PK model parameter estimates. A limited number of samples, as in phase II studies, may lead to missing a true association when EBE are used as phenotypes. Savic and Karlsson suggested a more extensive use of the likelihood ratio test (LRT) for covariate selection when \( \eta \)-shrinkage is large, but Combes et al. showed that the power to detect a covariate effect is the same with a LRT or a simple correlation test on EBE. The effect of sample size can be distinctly observed in our simulations. In the context of phase I studies, where the number of subjects is limited, the probability to detect the genetic effects was low, in line with our previous results. The combined analysis of phase I and phase II data allowed a marked improvement in this detection probability, irrespective of the phase II study design. By modifying the design of the phase II data, we highlighted a direct link between \( \eta \)-shrinkage, loss of genetic signal and probability to detect genetic variants. Our results showed that poor PK information due to the phase II study design results in higher \( \eta \)-shrinkage, which increases the loss of genetic signal at the estimation step and translates to a lower probability to detect genetic variant. The dilution of the individual information by adding subjects with sparse designs to subjects with rich designs increases as expected the loss of genetic signal. But this is accompanied with a sharp increase in detection power thanks to a larger sample size. \( \eta \)-shrinkage may also modify the EBE-EBE relationship, falsely inducing or masking correlations between model parameters. This could result in an increased number of false positives associated to other parameters than
CL, although in our simulations the number of FP on CL, V2 and Q remained of a similar magnitude across scenarios (Supplementary Table S3), showing no systematic effect.

We assume homogeneity of the PK between subjects simulated for the phase I and the phase II study. In practice healthy volunteers are often included in phase I while phase II studies focus on patients. A difference in typical values, for example of CL, between the two populations should not impact the detection power by combination of data, as the association tests use the phenotype variance, provided that the genetic effect is the same and that the model accounts for the systematic difference between clearances. It is more difficult to predict what would happen if the variability of clearance is different in the two populations, as the magnitude of the shrinkage in each subpopulation could affect the signal detection. When the assumption that the two populations are similar breaks down, we would suggest instead to combine rich and sparse data within the phase II study. Pharmacogenetic studies including a large number of subjects combining sparse and rich designs have already been published\textsuperscript{20,21}, showing that the combination of different sampling designs is feasible within the same study to assure more homogeneity.

Situations where pharmacogenetic analyses in PK studies are recommended are described by health authorities\textsuperscript{22}. In our work, we simulated a blinded pharmacogenetic analysis, exploring a large number of genetic markers. In real applications, other considerations than the statistical significance of genetic variants such as their physiological and clinical relevance could be factored in the analysis and its interpretation. Lehr et al.\textsuperscript{23} proposed in their stepwise procedure to select only significant polymorphisms having a physiologic relevance in the final model, and the same constraint could be integrated in penalised regression approaches. The probability to detect genetic variants could also be increased through the targeted inclusion of subjects for a few polymorphisms of interest, but this approach requires hypotheses on which polymorphisms to test, with a risk to miss important associations. We focused in this work on PK variability, which is a part of the variability in drug response. But the conclusions from the simulation study could be extended to pharmacodynamics. A previous survey indicated that most pharmacogenetic analyses in clinical PK studies used a phenotype estimated by NCA and furthermore included a limited number of subjects (lower than 50 subjects in two thirds)\textsuperscript{6}. Authorities in fact recommend to study pharmacogenetics in phase I\textsuperscript{22}, where the number of subjects is limited. Our work shows that such analyses do not have the power to detect polymorphisms efficiently but can
generate hypotheses to assess in later studies. A recent simulation work\textsuperscript{24} studied the sample size required to detect a binary covariate. They conclude that around 60 subjects combining rich or sparse designs was sufficient to detect the covariate with at least 80% power. Again our simulations showed that genetic covariates require higher sample sizes because they are highly unbalanced.

In the first series of simulations a moderate IIV on CL (25%) was used, resulting in a low impact of the genetics on PK, since overall 30% of the moderate CL variability was explained by genetic variants. This setting represented a realistic case to challenge the detection of genetic variants through modelling. We also evaluated the same scenarios with a higher IIV for CL, set to 60%. The $\eta$-shrinkage was much lower, as a higher IIV downweights the population priors in the combined criterion used to compute EBE. This decrease in CL $\eta$-shrinkages resulted in lower signal loss because of the direct relationship between the two. Associated with larger simulated effect sizes, the number of TP and the probability to detect genetic variants increased in these scenarios. The effect of $\eta$-shrinkage on the probability to detect genetic effects was in these simulations higher than the one we observed with the main settings, because the decrease of $\eta$-shrinkage was associated with a sharp increase in the number of TP. This shows that our conclusions don’t depend on the level of IIV.

This simulation study also confirms the results of our previous work concerning the relative performance of the different association methods\textsuperscript{6}. The penalised regression method Lasso and the stepwise procedure showed a similar probability to detect genetic variants in all scenarios. However, the Lasso is a slightly more complex method which requires computing the penalty in a first step before testing the associations. In this work we assessed methods to detect genetic effects on EBE, after an initial fit. An algorithm proposed by Lehr et al.\textsuperscript{23} uses univariate regressions to select variants to test in the PK model through LRT. This approach is easy to implement but run-times depend on the number of iterations leading to the full covariates model. An alternative is to use an integrated approach where effect sizes are estimated and significant variants selected using a penalised regression in the same step\textsuperscript{17}; it showed similar performance than the stepwise procedure proposed by Lehr et al., but with longer computing times\textsuperscript{17}. The results for the two other penalised regression methods tested in the previous work, ridge regression and HyperLasso, were similar (Supplementary Tables S7-S9, Figures S9-S10). None of the methods detected the 6 SNPs simultaneously, as 3 of the polymorphisms only explained 1 to 3% of the clearance
variability, making them difficult to detect. Because association methods relate the polymorphisms to the phenotype variance, we fixed the variance explained by the causal variants (through the parameter $R_{GC}$) and computed the effect sizes as a function of their allelic frequencies. For a given $R_{GC}$ an infrequent polymorphism was therefore associated with higher effect sizes. This reflects that a clinically relevant polymorphism (with a high impact on PK), present in few subjects because of its low frequency, will explain a limited proportion of the phenotype variance. Detecting such polymorphisms is crucial to identify subpopulation at risk but required much larger sample sizes, as in genome-wide studies. As an example, the rs3918290 polymorphism from gene DPYD has a frequency lower than 1%, but results in a deficient dihydropyrimidine dehydrogenase activity associated with a 40% decrease of the maximum conversion capacity of the chemotherapeutic drug 5-fluorouracil, resulting in severe toxicities.

The power to detect polymorphisms is also closely related to the type I error chosen for the analysis. In a context of exploratory analyses, we fixed the global type I error to 20%. But using the Sidak correction the significance thresholds were finally lower than 0.1% for each test, so that only strong effects of causal variants will be detected, and our simulations show that polymorphisms explaining a limited part of the phenotype variance are not detected. Approaches based on FWER and corrections as Bonferroni or Sidak are easy to implement but are conservative and may reduce the power of analyses, but limit the number of polymorphisms to test in later confirmatory trials. In practice other corrections for type I error could be considered, as permutation methods which are more time consuming but less conservative.

Although this correction was conservative and was calibrated under $H_0$ to control the FWER, the proportion of FP under $H_1$ amongst selected variants was higher than the expected 20%. This could reflect the correlations between polymorphisms we simulated.

To make more specific recommendations for study designs is difficult because it is closely related to the developed drug. In our simulations a late sample allowed larger information on the elimination phase to estimate CL. This result can be generalised to pharmacogenetic studies involving clearance and drugs with a long half-life. Taking a late sample requires to suspend treatment long enough to observe a decrease in concentrations, which may not be possible in patients from phase II trials.
In any case, it is essential that the sampling protocol, although limited, is as informative as possible to minimise the estimation error and shrinkage in individual parameters estimation. The detection of genetic polymorphisms could highly benefit from the use of larger sample sizes through combined analysis and optimised design\textsuperscript{18,27}.

In conclusion, this work confirmed the very limited likelihood that weak genetic effects can be detected in a typical phase I study, due to the small sample size. Such studies have to be considered only as hypothesis generating\textsuperscript{28}. On the basis of our results in term of detection probability when analysing together data from phase I and phase II study, we claim that phase II is the best moment to identify the impact of genetic variants on drug response. It would be less efficient to start the study of the pharmacogenetics of a new drug in phase III trials or in post-marketing, because these take place too late in drug development\textsuperscript{29} and the new treatment could be administered in non-responders or expose subjects to high toxicities. Furthermore genetic subpopulations can be better targeted and potentially some subjects excluded from the study to increase the efficacy and reduce the risk of toxicity of the drug in these phase III studies.

STUDY HIGHLIGHTS

What is the current knowledge on the topic?
Most pharmacogenetic analyses in pharmacokinetic studies recently published included a limited number of subjects (less than 50). Previous simulations showed that such sample sizes result in low probability to detect polymorphisms. But with large number of subjects, extensive pharmacokinetic information is difficult to obtain in drug development.

What question did this study address?
This simulation study explored realistic ways to increase the amount of information by combining rich phase I data and sparse phase II data, and optimising such sparse designs.

What this study adds to our knowledge?
This study shows that even sparse data from phase II allow a marked improvement in the probability to detect genetic variants when combined with rich data from phase I, even more when sparse designs are optimised.

How this might change clinical pharmacology and therapeutics?
The pharmacogenetic analyses should be planned later in the drug development to take advantage of larger sample sizes by combining data which would increase the power to detect genetic effects.

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Conflict of Interest
Adrien Tessier has a research grant from Institut de Recherches Internationales Servier and the French government. Marylore Chenel works for Institut de Recherches Internationales Servier, heading the department of Clinical Pharmacokinetics and Pharmacometrics.

Author Contributions
Adrien Tessier, Julie Bertrand, Marylore Chenel and Emmanuelle Comets designed the research. Adrien Tessier performed the research. Adrien Tessier, Julie Bertrand, Marylore Chenel and Emmanuelle Comets analyzed the results. Adrien Tessier, Julie Bertrand, Marylore Chenel and Emmanuelle Comets wrote the manuscript.

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TABLES

Table 1. Population values (µ) and interindividual variability (ω) for the model parameters of drug S used in the simulation study.

| Parameters | µ   | ω (%) |
|------------|-----|-------|
| F<sub>a</sub> | Imax<sub>F</sub> | 0.8   | 32.9  |
|            | D50<sub>F</sub>  | 41.7  |       |
| FRAC<sub>b</sub> | Emax<sub>FRAC</sub> | 0.45  |       |
|            | D50<sub>FRAC</sub>| 18.6  |       |
| Tlag<sub>1</sub> | 0.401 | 35.1  |       |
| Tk0        | 1.59  | 31.6  |       |
| Tlag<sub>2</sub> | 22.7  |       |       |
| Ka         | 0.203 |       |       |
| V1         | 1520  |       |       |
| V2         | 2130  | 44.2  |       |
| CL         | 94.9  | 25.1  |       |
| σ<sub>slope (%)</sub> | 20    |       |       |

a. For doses < 20 units F = 1, for doses ≥ 20 units F = 1 - \( \frac{Imax_F(dose - 20)}{D50_F + dose - 20} \), where dose is the amount administered.

b. \( FRAC = \frac{Emax_{FRAC} \cdot dose}{D50_{FRAC} + dose} \)

F: bioavailability; FRAC: fraction of dose; Tk0: zero order absorption duration; Tlag<sub>1</sub>: lag time of zero order absorption; Ka: first order absorption constant rate; Tlag<sub>2</sub>: lag time of first order absorption; V1: central compartment volume; V2: peripheral compartment volume; Q: intercompartmental clearance; CL: linear elimination clearance.

Table 2. Empirical estimates of Family-Wise Error Rate under H<sub>0</sub> for both association tests.

| Method               | FWER (%) | SPI | SPI/I1<sub>1.96h</sub> | SPI/I1<sub>3.24h</sub> | SPI/I1<sub>15.24h</sub> |
|----------------------|----------|-----|------------------------|----------------------|------------------------|
| Lasso                | Without correction<sup>a</sup> | 14  | 17.5                   | 21.5                 | 13.5                   |
| Stepwise procedure   | Without correction<sup>a</sup> | 20  | 18.5                   | 22.5                 | 15.5                   |
| Lasso                | After empirical correction<sup>b</sup> | 20  | 19.5                   | 21.5                 | 19.5                   |
| Stepwise procedure   | After empirical correction<sup>b</sup> | 20  | 20.5                   | 22.5                 | 20.5                   |

a. Set of empirical family wise error rates (FWER) obtained without correction.
b. Set of empirical FWER obtained after correction of type I error per tests.

The 95% prediction interval around 20 for 200 simulated datasets is [14.5-25.5].
**Figure 1.** Workflow of the simulation study divided in the simulation (blue box) and analysis part (red box).

- a. at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 120 and 192h.

- $CL_{\text{sim}}$: simulated individual clearance ($i = 1, ..., N = N_1 + N_2$); $EBCl_{\text{sim}}$: empirical Bayes estimate of clearance; $H_0$: null scenarios; $H_1$: alternative scenarios; $FWER$: family wise error rate; $N_1$: number of subjects from the phase I study; $N_2$: number of subjects from the phase II study; $N_t$: number of polymorphic SNP to analyse; $R_{GC}$: genetic component of the interindividual variability; $SNP_{ik}$: single nucleotide polymorphism ($k = 1, ..., N_t$); $p$: p value; $p$: correlation coefficient between variants; $\alpha$: type I error per test; $\beta_k$: effect size coefficient; $\xi$: Lasso tuning parameter.

\[
\xi = \Phi^{-1}\left(1 - \frac{\alpha}{N_t}ight) \sqrt{\frac{N_1 + N_2}{N}}
\]

\[
\beta_k = \arg \min_{\beta_k} \sum_{i=1}^{N_1} \left( \log(EBCl_{\text{sim}}) - \sum_{k=1}^{N_t} \beta_k \cdot SNP_{ik} \right)^2 + \xi \sum_{k=1}^{N_t} |\beta_k|
\]
Figure 2. True Positive Rate (TPR) versus False Positive Rate (FPR) under $H_1$ (top) and probability estimates (points) and 95% confidence interval (bars) to detect at least $x$ causal variants explaining the interindividual variability of CL ($x = 1, ..., 6$) under $H_1$ (bottom) for main scenarios simulated with $IIV_{CL} = 25\%$ (left) or modified scenarios simulated with $IIV_{CL} = 60\%$ (right). Different symbols are used for each scenario, and colours denote the Lasso (grey) and the stepwise procedure (light blue).
Figure 3. Distribution of the $\eta$-shrinkages on clearance for subjects in the phase I dataset (blue) and for subjects in the phase II dataset (brown), for each main scenario simulated under $H_0$ with IIV $\text{CL} = 25\%$. 
Figure 4. Boxplot showing the loss of the signal for genetic effect in the overall population (top), as well as separately for the phase I data (blue borders) and for the phase II data (brown borders) (bottom). A boxplot is shown separately for each main scenario simulated under H₁ with IIV_{CL} = 25% as a function of increasing R_{GC} (boxplots colour).