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ORIGINAL ARTICLE

# Utility of Integrated Analysis of Pharmacogenomics and Pharmacometabolomics in Early Phase Clinical Trial: A Case Study of a New Molecular Entity

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In this report, we present a case study of how pharmacogenomics and pharmacometabolomics can be useful to characterize safety and pharmacokinetic profiles in early phase new drug development clinical trials. During conducting a first-in-human trial for a new molecular entity, we were able to determine the mechanism of dichotomized variability in plasma drug concentrations, which appeared closely related to adverse drug reactions (ADRs) through integrated omics analysis. The pharmacogenomics screening was performed from whole blood samples using the Affymetrix DMET (Drug-Metabolizing Enzymes and Transporters) Plus microarray, and confirmation of genetic variants was performed using real-time polymerase chain reaction. Metabolomics profiling was performed from plasma samples using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *A GSTM1* null polymorphism was identified in pharmacogenomics test and the drug concentrations was higher in *GSTM1* null subjects than *GSTM1* functional subjects. The apparent drug clearance was 13-fold lower in *GSTM1* null subjects than *GSTM1* functional subjects ($p < 0.001$). By metabolomics analysis, we identified that the study drug was metabolized by cysteinylglycine conjugation in GSTM functional subjects but those not in *GSTM1* null subjects. The incidence rate and the severity of ADRs were higher in the *GSTM1* null subjects than the *GSTM1* functional subjects. Through the integrated omics analysis, we could understand the mechanism of inter-individual variability in drug exposure and in adverse response. In conclusion, integrated multi-omics analysis can be useful for elucidating the various characteristics of new drug candidates in early phase clinical trials.

**Keywords:** clinical trial, metabolomics, new drug development, pharmacogenomics

## Introduction

Improving efficiency is an important issue in new drug development. Although there have been considerable advances in science and technology, the number of approved new drugs per research spending has continuously declined over 60 years [1]. Dramatic improvements were made in pre-clinical research areas, but the successful clinical development rate of new drug candidates has not changed in recent years [1]. To improve efficiency in clinical trials, enrichment strategies were proposed in several disease areas, which enable precision medicine in drug development [2-4].

Various omics technologies have been used in recent clinical trials to elucidate the various characteristics of new drug candidates and to identify optimal subjects for the trials [5-10]. Pharmacogenomics is used in clinical trials to select appropriate patients and to further explain pharmacokinetics, pharmacodynamics, efficacy, and adverse drug reactions of new drug candidates [6-8]. Pharmacometabolomics can be used in clinical trials to identify drug targets, diagnosis disease, assess drug metabolic enzyme

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function in humans, and monitor the drug response and toxicity [9-13]. The importance of those evaluations is high in early phase clinical trials because it can help to simplify late phase clinical trials and increase the probability of success of clinical trials [5].

In this report, we present a case showing how pharmacogenomics and pharmacometabolomics were used in an early phase new drug development clinical trials. Drug X is a transient receptor potential vanilloid type 1 (TRPV1) antagonist class of new drug candidate that is under clinical development for the treatment of atopic dermatitis. In a first-in-human clinical trial for drug X, large inter-individual variability in the plasma concentration was observed after a single oral administration. Furthermore, moderate adverse drug reactions were observed in certain group of subjects. To perform subsequent clinical trials safely and efficiently, we needed to understand the cause of the inter-individual variability observed in the early phase clinical trial for drug X.

The aim of this study was to investigate the mechanism of the variability in plasma concentration of drug X and the adverse drug reaction by integrated multi-omics analysis using pharmacogenomics and pharmacometabolomics tools.

## Methods

### Study samples

The first-in-human clinical study enabled exploratory analysis of pharmacogenomics and pharmacometabolomic analysis. The study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea (H-1011-027-339) and was conducted in accordance with the principles of the Declaration of Helsinki and ICH Good Clinical Practice. All subjects provided written informed consent before any study-related procedure was performed.

Whole blood samples (n = 32) were obtained from every subject who received a single oral dose of drug X for the

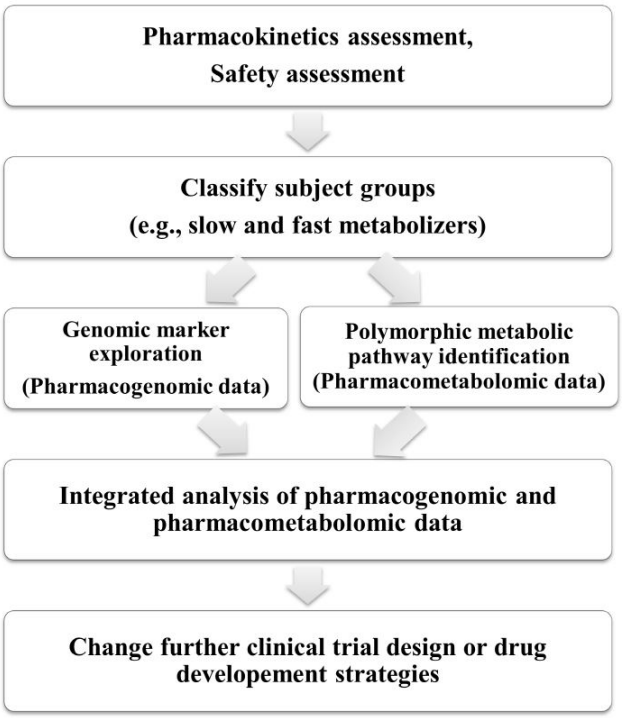


**Fig. 2.** Study flow.

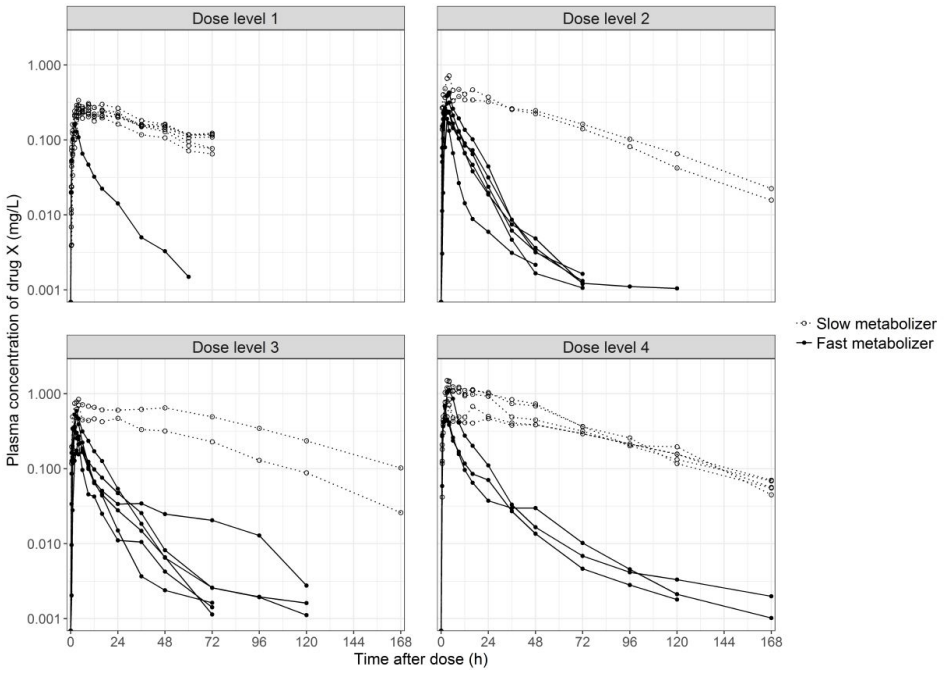


**Fig. 1.** Plasma concentration-time profiles of drug X after single oral administration.

pharmacogenomic genotyping. Plasma samples (n = 8) were obtained from the subjects who received highest dose of drug X for the pharmacometabolomic analysis.

### Genotyping

Genomic DNA was extracted from the whole blood using a QIAamp DNA Mini Kit (QIAgen, Hilden, Germany). The pharmacogenomics screening was performed using an Affymetrix DMET (Drug-Metabolizing Enzymes and Transporters) Plus microarray (Affymetrix, Santa Clara, CA, USA) at DNA Link Co. Ltd. (Seoul, Korea) according to the previously described method [14]. The microarray covered 1,931 single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels) and 5 copy-number variations for 255 genes involved in the metabolism, transport, and excretion of drugs in humans and its utility and robustness has been validated in previous reports [15, 16]. Furthemore, a real-time polymerase chain reaction (PCR) was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) to confirm the *GSTM1* copy number variant identified from the microarray.

### Pharmacometabolomic analyses

Metabolomic profiling to identify metabolites of drug X was performed in plasma samples using liquid chromatography (Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA) coupled with quadrupole time-of-flight mass spectrometry (Agilent 6530 Q-TOF MS, Agilent Technologies). To prepare the analyte, a 100 $\mu$L of plasma sample was mixed with 400 $\mu$L of acetonitrile. Five microliters of supernatant aliquot was injected onto the Agilent Zorbax C18 column (1.8 $\mu$m particle size, 2.1 × 50 mm; Agilent Technologies) at 30°C under gradient elution for 18 min. The mobile phase consisted of a mixture of 0.1% formic acid in 2 mM ammonium formate and 0.1% formic acid in acetonitrile. Positive ion electrospray ionization mode was used for mass spectrometry. The phase I and phase II metabolites of drug X were identified by molecular feature extraction of Agilent MassHunter Qualitative Software version B.03.01 (Agilent Technologies).

### Data analysis

The pharmacokinetic parameters of drug X were analyzed by noncompartmental analysis and presented using descriptive statistics. The maximum plasma concentration ($C_{max}$) and the area under the plasma concentration-time curve from 0 to the last measurable concentration ($AUC_{last}$), which represents the systemic exposure to drug x, is summarized by genotype and dose groups. The apparent clearance (CL/F), which represents the capacity of drug elimination from the body, was pooled among the dose groups, and the Mann–Whitney U test was used to compare the CL/F between the genotype groups. A p-value of $<0.05$ was considered to be statistically significant. The number of drug-related adverse events (AEs) between the genotype groups was compared using the chi-square test and Fisher's exact test. Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

## Results

### Subject classification and study flow

After pharmacokinetics and safety assessment of the original first-in-human clinical study, the subjects were clearly classified into two groups, namely, slow and fast metabolizers (Fig. 1). After the classification, genomic marker exploration was performed by pharmacogenomic analysis and metabolic pathway identification was

**Table 1.** Pharmacokinetic parameters of drug X following single oral dose

| Parameters | Dose level 1 | | Dose level 2 | | Dose level 3 | | Dose level 4 | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *GSTM1* (−/−) | *GSTM1* (−/+) or (+/+) | *GSTM1* (−/−) | *GSTM1* (−/+) or (+/+) | *GSTM1* (+/−) | *GSTM1* (−/+) or (+/+) | *GSTM1* (−/−) | *GSTM1* (−/+) or (+/+) | *GSTM1* (−/−) | *GSTM1* (−/+) or (+/+) | p-value |
| No. | 7 | 1 | 2 | 6 | 2 | 6 | 5 | 3 | 16 | 16 | - |
| CL/F (L/h) | 1.1 ± 0.3 | 14.6 | 1.8 ± 0.0 | 21.6 ± 12.2 | 2.1 ± 1.0 | 30.6 ± 11.5 | 3.3 ± 0.8 | 30.1 ± 10.8 | 2.0 ± 1.1 | 26.1 ± 11.7 | <0.001 |
| $C_{max}$ (mg/L) | 0.27 ± 0.04 | 0.16 | 0.57 ± 0.21 | 0.28 ± 0.09 | 0.78 ± 0.09 | 0.37 ± 0.15 | 1.14 ± 0.36 | 0.74 ± 0.35 | - | - | - |
| $AUC_{last}$ (mg · h/L) | 11.87 ± 1.47 | 1.35 | 26.85 ± 0.39 | 2.73 ± 1.08 | 51.82 ± 23.63 | 3.61 ± 1.33 | 59.72 ± 14.10 | 7.33 ± 3.26 | - | - | - |
| $t_{1/2}$ (h) | 47.0 ± 12.7 | 13.4 | 32.0 ± 0.0 | 69.6 ± 28.5 | 35.0 ± 7.8 | 58.6 ± 28.0 | 38.9 ± 6.9 | 51.2 ± 13.8 | - | - | - |

Values are presented as mean ± standard deviation.
GSTM1(−/−), subject with glutathione-S-transferase M1 null function; GSTM1(−/+) or (+/+), subject with one or two glutathione-S-transferase M1 functional allele; CL/F, apparent clearance; $C_{max}$, maximum plasma concentration; $AUC_{last}$, area under the plasma concentration-time curve from 0 to the last measurable concentration; $t_{1/2}$, terminal elimination half-life.

performed by drug pharmacometabolomic analysis. The genomic marker data was integrated with metabolic pathway data to understand the mechanism of the inter-individual variability. The overall study flow is presented in Fig. 2.

## Pharmacogenomics analysis results

In the slow metabolizer subjects, homozygous deletion of the glutathione-S-transferase M1 gene (*GSTM1* null), which leads to loss of enzyme function, was identified from the DMET Plus microarray. The systemic exposure to drug X, which is represented by the $C_{max}$ and the $AUC_{last}$, was higher in *GSTM1* null subjects than *GSTM1* functional subjects in every dose group (Table 1, Fig. 2). The apparent drug clearance (CL/F) was 13-fold lower in *GSTM1* null subjects than *GSTM1* functional subjects ($p < 0.001$) (Table 1). The *GSTM1* deletion polymorphism was confirmed using real-time PCR, and subjects with two functional *GSTM1* alleles showed similar drug exposure to subjects with one functional *GSTM1* allele (Fig. 3).

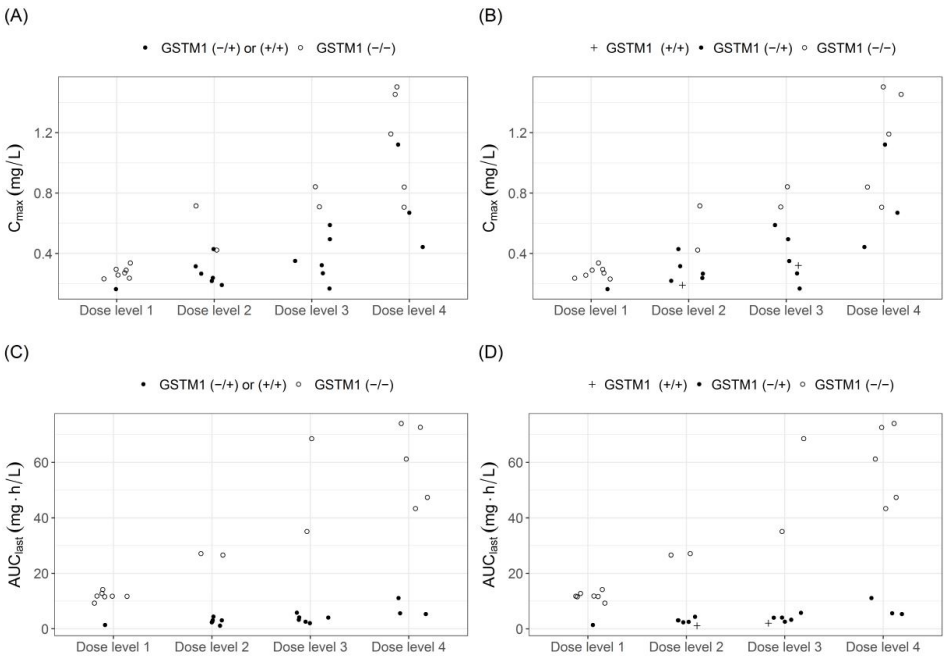


**Fig. 3.** (A–D) Comparison of the pharmacokinetic parameters of drug X among the different *GSTM1* genotype groups. *GSTM1*(−/−), subject with glutathione-S-transferase M1 null function; *GSTM1*(−/+), subject with one glutathione-S-transferase M1 functional allele; *GSTM1*(+/+), subject with two glutathione-S-transferase M1 functional allele; $C_{max}$, maximum plasma concentration; $AUC_{last}$, area under the plasma concentration-time curve from 0 to the last measurable concentration.

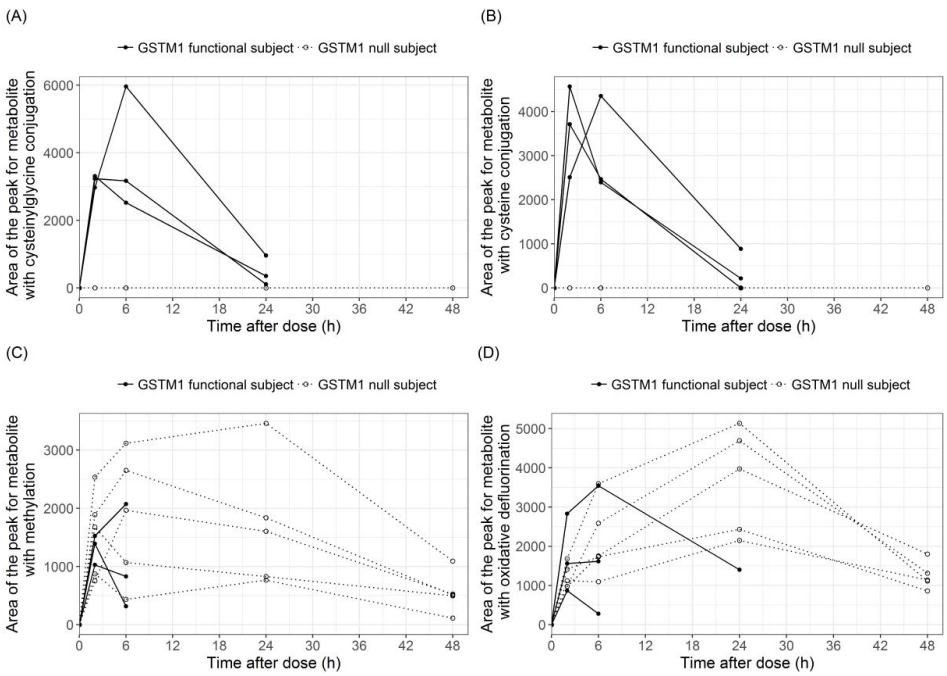


**Fig. 4.** (A–D) Plasma drug X metabolite-time profiles after single oral administration.

### Pharmacometabolomic analyses result

A total of four metabolites of drug X (cysteine conjugation, cysteinylglycine conjugation, methylation, oxidative defluorination) were observed from the plasma samples. Among these metabolites, cysteine and cysteinylglycine conjugation metabolites were detected in functional *GSTM1* subjects, but they were absent in *GSTM1* null subjects (Fig. 4). The areas under the peak for methylation and oxidative defluorination metabolites were higher in the *GSTM1* null subjects than the functional *GSTM1* subjects (Fig. 4).

### *GSTM1* null genotype and drug-related AE

The number of drug-related AEs and the number of subjects with drug-related AEs were higher in the *GSTM1* null subjects than the *GSTM1* functional subjects, although it failed to reach statistical significance (Table 2). Furthermore, the AEs were more severe in the *GSTM1* null subjects than the *GSTM1* functional subjects. Three subjects in the *GSTM1* null group experienced AEs with moderate severity, whereas none in the *GSTM1* functional group experienced those AEs. Those AEs were feeling hot, feeling cold and a burning sensation, and these sensations are frequently reported AEs in TRPV1 antagonist class drugs [17, 18].

## Discussion

Through the integrated omics analysis, we could understand the reason for the inter-individual pharmacokinetic variability observed in the first-in-human trial for drug X. The GSTs are a group of phase II enzymes that play an important role in the biotransformation of various xenobiotic and endogenous compounds by conjugation with glutathione [19]. *GSTM1* is a $\mu$ class isoform of GST, and the *GSTM1* gene is highly polymorphic in humans. Homozygous deletion of the *GSTM1* gene is observed in various ethnic groups, and its frequency ranged from 29.6% to 56.2% [20, 21]. The frequency of *GSTM1* null subjects was 50% in this study, consistent with the previously reported *GSTM1* null frequency in Koreans [20]. The loss of catalytic function is suggested to be reason for decreased systemic clearance of drug X in *GSTM1* null subjects. This hypothesis was supported by the metabolite analysis showing that no cysteine conjugation or cysteinylglycine conjugation metabolites of drug X were observed in *GSTM1* null subjects.

The effect of the *GSTM1* null polymorphism on systemic exposure to drug X was reproduced in subsequent clinical trials. The *GSTM1* null subjects showed higher plasma concentration and lower CL/F of drug X than functional

**Table 2.** Summary of drug-related AEs

| | *GSTM1* null subject (n = 16) | *GSTM1* functional subject (n = 16) | p-value |
|---|---|---|---|
| No. of drug-related AEs | 26 | 16 | 0.123[a] |
| No. of subjects with drug-related AEs | 9 (56.3) | 6 (37.5) | 0.288[a] |
| No. of subjects with mild AEs | 8 (50) | 6 (37.5) | 0.476[a] |
| No. of subjects with moderate AEs | 3 (18.8) | 0 | 0.226[b] |
| No. of subjects with severe AEs | 0 (0) | 0 | - |

Values are presented as number (%).
AE, adverse event.
[a]Chi-squared test; [b]Fisher's exact test.

**Table 3.** Pharmacokinetic parameters of drug X following multiple oral doses

| Parameters | Dose level 5 | | Dose level 6 | | Total | | |
|---|---|---|---|---|---|---|---|
| | *GSTM1* (–/–) | *GSTM1* (–/+) | *GSTM1* (–/–) | *GSTM1* (–/+) | *GSTM1* (–/–) | *GSTM1* (–/+) | p-value |
| No. | 7 | 1 | 5 | 3 | 12 | 4 | |
| $CL/F_{ss}$ (L/h) | 0.4 ± 0.1 | 10.4 | 0.4 ± 0.1 | 7.5 ± 4.6 | 0.4 ± 0.1 | 8.2 ± 4.0 | 0.001 |
| $C_{max,ss}$ (mg/L) | 0.23 ± 0.03 | 0.06 | 0.47 ± 0.14 | 0.15 ± 0.04 | - | - | - |
| $AUC_{\tau,ss}$ (mg · h/L) | 4.25 ± 0.54 | 0.41 | 8.26 ± 1.85 | 1.22 ± 0.52 | - | - | - |
| $t_{1/2,ss}$ (h) | 36.3 ± 4.7 | 42.0 | 37.2 ± 3.1 | 72.3 ± 3.4 | - | - | - |

Values are presented as mean ± standard deviation.
GSTM1(–/–), subject with glutathione-S-transferase M1 null function; GSTM1(–/+), subject with one glutathione-S-transferase M1 functional allele; $CL/F_{ss}$, apparent clearance at steady state; $C_{max,ss}$, maximum plasma concentration at steady state; AUCτ,ss, area under the plasma concentration-time curve within a dosing interval at steady state; $t_{1/2,ss}$, terminal elimination half-life at steady state.

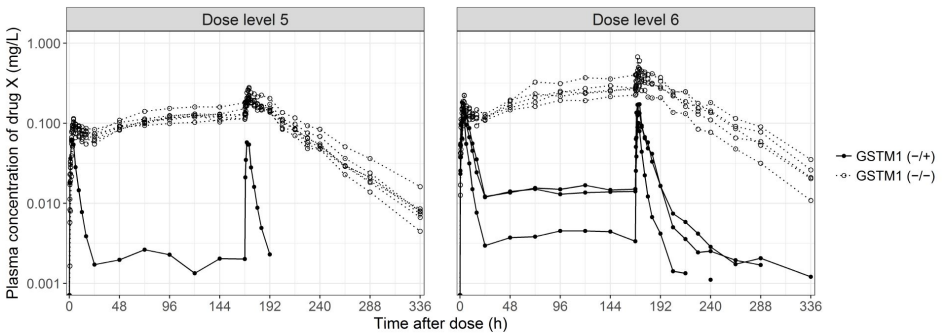


**Fig. 5.** Plasma concentration-time profiles of drug X after multiple oral administration. *GSTM1*(−/−), subject with glutathione-S-transferase M1 null function; *GSTM1*(−/+), subject with one glutathione-S-transferase M1 functional allele.

*GSTM1* subjects after multiple oral administrations (Table 3, Fig. 5). The clinical development strategy for drug X needed to be modified to reflect these results because the *GSTM1* null variant was a major determining factor for drug exposure, as well as drug-related AEs, and because the frequency of *GSTM1* nulls is approximately half of total population. A personalized drug development strategy applying different oral doses based on the *GSTM1* genotype or switching to a different formulation with limited systemic exposure was needed for safe and efficient development of drug X. drug X is currently in clinical development as a topical formulation to limit systemic absorption.

Integrated multi-omics analysis can be a useful tool to evaluate the diverse characteristics of new drug candidates in clinical situations. The importance of integration of multi-omics data is growing in many research areas, including pharmaceutical research and development [22-24]. As shown in this case study, integrated multi-omics analysis can be used to explain the cause of inter-individual variability in drug exposure and adverse drug reactions observed in clinical development. This information can be important when making decisions for further drug development strategy.

In conclusion, integrated multi-omics analysis can be useful in early phase clinical trials for elucidating the various characteristics of new drug candidates that could not be produced through traditional clinical trial methods.

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## Authors' contribution

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Data curation: SY, NG, DS
Formal analysis: SY, JO
Funding acquisition: I-JJ
Methodology: SHY, J-YC
Writing – original draft: JO, SHY
Writing – review & editing: SY, NG, DS, K-SY, J-YC, I-JJ

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