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Discovering CYP2D6 activity biomarkers by correlating MDMA pharmacokinetic parameters to untargeted metabolomics data – A proof of concept study

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1. Background

Cytochrome P450 (CYP) enzymes

- Most relevant enzymes in drug metabolism
- Huge inter-individual variation in activity which is not exclusively attributable to individual's genotype

Metabolomics

- Metabolomics: study of endogenous metabolites
- Aim: discover biomarkers indicative of phenotype

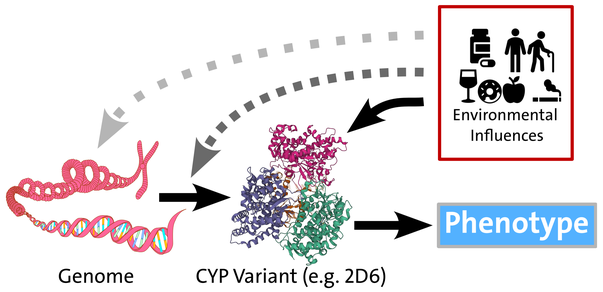


Fig.1: CYP genotype ≠ phenotype due to environmental influences on enzymes functionality (➞), (post)translational or direct influence on the genetic code (⇢/⇢).

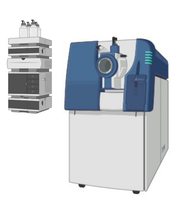
2. Methods (I)

Sample preparation

- Plasma samples diluted and protein precipitated (MeOH/Aceton, 9:1; 50 µL sample, 150 µL solvent)

LC-MS/MS

- LC-qTOF-MS using a Sciex TripleTOF 6600
- Untargeted DDA method



Data analysis

- msDial 4.8 for peak picking
- R statistical language further statistical analysis
- Spearman correlation analysis

2. Methods (II)

Modulation approach

- Challenge to recruit all CYP isoform phenotypes
- A smaller group could undergo modulation approach shown in Fig. 2.[1]

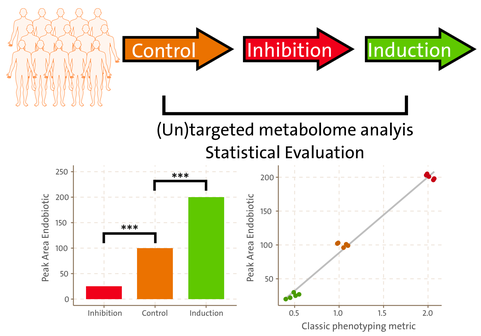


Fig.2: Modulation approach: phenotype artificially changed by either inhibition/induction through drugs[1].

Correlation

- Metabolomics approach compares poor/intermediate metabolizers with extensive metabolizers.
- Our approach uses a CYP phenotype metric using MDMA pharmakokinetic data[2] (Eq. 1).

$$\text{CYP phenotype metric} = \frac{\text{AUC}[\text{MDMA}_{\text{Metabolites}}]}{\text{AUC}[\text{MDMA}]} \quad (1)$$

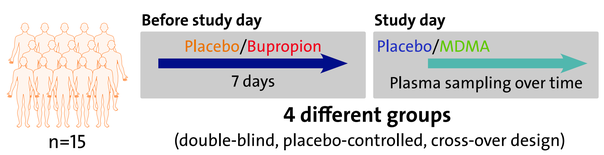


Fig.3: The metabolites of bupropion inhibit CPY2D6 which generates functional poor/intermediate metabolizers. MDMA (CYP2D6 substrate) kinetic data was used for correlation.

3. Results

- Correlation analysis yielded **122 features of interest**
- **5 identified to be bupropion** (or closely related), **25 with chlorine isotope patterns** potentially exogenic
- After manual curation of peaks and correlation (shape, visual inspection) **61 features of interest** (example of correlation plot shown in Fig. 4)
- **2 of those features identified** by reference substance (Fig. 5) as potential endogenous CYP2D6 biomarkers.

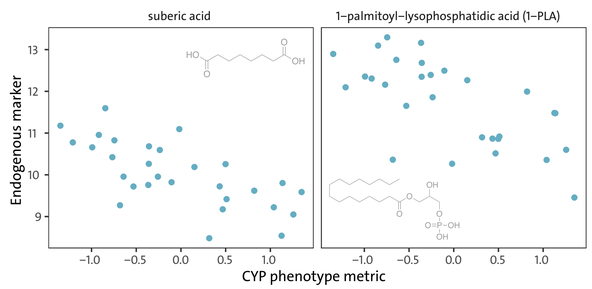


Fig.4: The y axis labelled with "Endogenous marker" shows the peak area for the respective features (left: suberic acid; right: 1-PLA) while the x axis shows the ratio of the area under the curve (AUC) of all MDMA metabolites and the AUC of MDMA (Eq. 1). Both axis are logarithmically scaled.

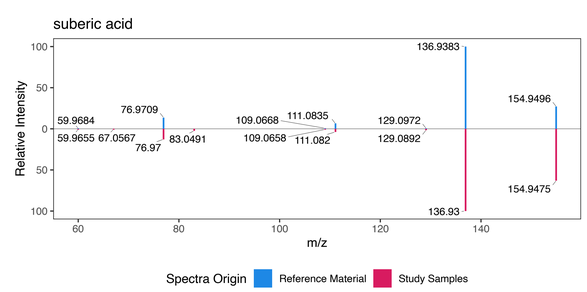


Fig.5: Spectra from reference material in blue; study samples spectra in magenta.

Conclusion

- Correlating the pharmacokinetic parameters of MDMA (CYP phenotype metric, Eq. 1) to unknown metabolomics features leads to promising results
- Two new potential endogenous CYP2D6 phenotype markers, suberic acid and 1-PLA, were identified using this methodology.
- New endogenous markers could greatly improve toxicological expert reporting, although bigger cohorts and studies are needed to confirm these.

4. Outlook

- Further studies needed to confirm applicability of suberic acid and 1-PLA as endogenous phenotype biomarkers
- Identification of remaining promising but unknown features necessary
- Methodology needs further testing, especially in larger cohorts

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Disclosure: I declare that neither I, nor any member of my immediate family, have a financial interest in a company as defined in the AACC policies on conflict of interest or any other relevant conflict of interests.

References

1. G. Magliocco, A. Thomas, J. Desmeules, et al., Phenotyping of Human CYP450 Enzymes by Endobiotics: Current Knowledge and Methodological Approaches, Clin Pharmacokinet, Vol 58, pp. 1373-1391, **2019**.
2. A. E. Steuer, C. Schmidhauser, E. H. Tingelhoff, et al., Impact of Cytochrome P450 2D6 Function on the Chiral Blood Plasma Pharmacokinetics of 3,4-Methylenedioxymethamphetamine (MDMA) and Its Phase I and II Metabolites in Humans, PLOS ONE, Vol 11, p. e0150955, **2016**.