Population pharmacokinetic and pharmacogenetic analysis of mitotane in patients with adrenocortical carcinoma: towards individualized dosing

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Online Resource 1 Supplementary methods, figures and tables.

Contents

Supplementary population PK analysis methods

One-, two- and three-compartment models, with first-order absorption and first-order elimination, were explored as the structural model. Relative standard error (RSE) of parameters, which represent the precision of parameter estimates, and the objective function value (OFV) were considered when evaluating the structural models. The one with acceptable RSE and lower OFV was selected as the final basic model structure.

Inter-individual variability (IIV) of parameters were estimated with Eq. 1, where P_i represents the parameter of ith individual and was assumed to be log-normally distributed, P_t represents typical value of the parameter, and η_{IIV} represents the random IIV which was assumed to be normally distributed with mean of 0 and variance of ${\omega_1}^2$. In addition, inter-occasion variability (IOV), which reflects the intra-individual variability, of apparent systematic clearance (CL/F) was also included when analyzing the full dataset. As is shown in Eq.S1, η_{IOV} represents the random IOV. The distribution of η_{IOV} in each occasion was assumed to be similar and normally distributed with mean of 0 and variance of ${\omega_2}^2$. In this study, every 200 days of treatment was defined as an occasion as the total observation periods of the patients were long.

The residual error was characterized with a combined proportional and additive model as is shown in Eq. S2, where Obs represents observations, $IPRED$ represents individual predictions, and ε_1 and ε_2 represent the proportional residual error and additive residual error respectively which were assumed to be normally distributed with mean of 0 and variance of ${\sigma_1}^2$and ${\sigma_2}^2$, respectively.

$$P_i = P_t \cdot \mathrm{e}^{\eta_{IIVi}+\eta_{IOVj}} \qquad \text{Eq.S1}$$

$$Obs = IPRED \cdot (1 + \varepsilon_1) + \varepsilon_2 \qquad \text{Eq.S2}$$

As for the covariate analysis, the identified SNPs, as well as patients' demographic information and clinical characteristics were considered. For continuous covariates, for each patient the mean values of all measurements during the monitoring period were taken. In case of missing continuous covariates, the corresponding median value of all patients was assigned. For patients who only missed HT but not WT, LBW was calculated using real WT and imputed HT. For GFR, 0 (normal) was assigned if ≥ 50 %

of the collected patient's records were 0 otherwise 1 was assigned. Patients who missed GFR measurements, 0 was assigned.

The effect of all above covariates on mitotane CL/F and the effect of WT, LBW, FAT, and gender on apparent distribution volumes (V/F) were investigated using stepwise covariate modelling (SCM) function implemented with Perl-Speaks-NONMEM (version 4.7.0) [1]. Both a forward inclusion ($p < 0.05$) and a backward elimination process ($p < 0.01$) were performed to identify significant covariates. For SNPs that were in 100% linkage disequilibrium, if they were included during the SCM analysis, the more clinically relevant ones would be selected in the final model. The effects of continuous covariates were investigated with both linear relation (Eq.S3) and power relation (Eq.S4), where P_i represents the parameter of ith individual, P_t represents typical value of the parameter, and η_i represents the individual variability, θ_{COV} represents the estimate of covariate effect, COV_i represents the covariate value of ith individual, COV_m is the median value of the covariate. Categorical covariates were analyzed with Eq. S5, where θ_{COV} was set as 1 for reference category and was estimated for other categories.

$$P_i = P_t \cdot (1 \pm \theta_{COV} \cdot (COV_i - COV_m)) \cdot e^{\eta_i} \quad \text{Eq.S3}$$

$$P_i = P_t \cdot \left(\frac{COV_i}{COV_m}\right)^{\theta_{COV}} \cdot e^{\eta_i} \quad \text{Eq.S4}$$

$$P_i = P_t \cdot \theta_{COV} \cdot e^{\eta_i} \quad \text{Eq.S5}$$

Supplementary model evaluation methods

pcVPC was performed by 1000 times of simulation and the data points, 5th, 50th, and 95th percentiles of prediction-corrected observations were plotted together with 95% confidence intervals (CI) of 5th, 50th, and 95th percentiles of simulations. NPDE evaluation was performed with npde package (version 2.0) implemented in R statistics software based on 1000 times of simulations. The bootstrap was conducted by 1000 runs of bootstrap replicates sampled from original dataset with replacement, which was stratified on whether the subject contributed more than two data points after the end of treatment.

The median as well as 95% CI of parameters were derived and compared with original parameter estimates.

Supplementary simulation method

Based on the final model structure, simulations were performed to evaluate different designed treatment strategies and approaches of starting dose determination. Patients were assumed to receive treatment as long as their last mitotane concentration monitoring time. The blood samples were assumed to be collected once every 2 weeks after knowing the result of the last sample, and the concentration of mitotane was assumed to be known 7 days after blood collection, which is in accordance with the optimal scenario in the clinical practice. The dose amount was subsequently adjusted accordingly.

As a comparison, a previous recommended 'high-dose' starting regimens, where the mitotane dose starts with 1.5g per day and increases up to 6g per day in 4 days, were simulated (**Regimen 1**)[2].

As for the newly designed regimens, the starting dose was 1) set as 2g, 4g, or 6g for all patients according to the guideline[3] **(Regimen 2, 4, and 6**) or 2) set individually considering patients characteristics with the help of the model (**Regimen 3, 5, 7, and 8**). As the expected time to reach the therapeutic target of mitotane is 3 to 5 months, the individually starting daily mitotane dose was estimated as the dose that allows the predicted mitotane concentrations on day 98 (C_{sim_pred98}) reach the therapeutic target. The C_{sim_pred98} was obtained by performing simulation under a regimen of 6g per day increasing by 0g (**Regimen 8**), 0.5g (**Regimen 2, 3, 4, 5, 6-1, and 7-1**), or 1g (**Regimen 6-2 and 7-2**) once every 21 days till the 98th day of treatment, with only typical parameter values and covariate effects considered. Given the linear PK feature of mitotane, the suggested starting daily dose ($Dose$) was therefore determined by Eqs. S6 and S7, where $\lceil X \rceil$ represents the least integer greater than or equal to X, $\lfloor X \rfloor$ represents the greatest integer less than or equal to X. Determining the starting dose based on the C_{sim_pred} on day 77 and 119 were also used for comparison.

$$X = \frac{14\ \text{mg/L}}{C_{sim\_(i)pred}} \cdot 6\text{g} \qquad \text{Eq. S6}$$

$$Dose = \begin{cases} \lceil X \rceil, & X - \lfloor X \rfloor > 0.650 \\ \lfloor X \rfloor + 0.5, & 0.350 \leq X - \lfloor X \rfloor \leq 0.650 \\ \lfloor X \rfloor, & X - \lfloor X \rfloor < 0.350 \end{cases} \quad \text{Eq. S7}$$

Besides the above regimens, since individual parameters could be estimated after knowing one TDM result, **Regimen 9** was also designed and evaluated. In this strategy, patients were assumed to start with 4g per day until the first TDM result was obtained. C_{sim_real} of each patient on day 14 was simulated, based on which the η_{IIVi} and η_{IOV1} were estimated for each patient using NONMEM with the POSTHOC function. Subsequently, the next daily dose of each patient was determined with Eq. S6-S7 according to the individual C_{sim_pred98} ($C_{sim\_ipred98}$) under the daily dosing of 6g, based on the model incorporating η_{IIVi} as was suggested in a previous study[4]. The constant starting regimen was applied in this regimen.

In **Regimen 2 to 8**, the dose increasing amount when $C_{sim\_real} < 14$ mg/L was set differently before and after the target was reached (starting and maintenance regimen), in order to limit the toxicity at start and maintain the mitotane trough concentration within the therapeutic range at a later phase. The combination of 0g/1.5g, 0.5g/1.5g, 0.5g/1g, and 1g/1.5g were simulated and evaluated. **Regimen 2 to 7** applied stepwise increasing starting regimen and **Regimen 8** applied constant starting regimen. A maximum number of days that follows the starting regimen was set as 126 (around 4 months) and 105 (98+7 days) for the stepwise increasing or constant starting regimens, respectively.

When C_{sim_real} reached 20 mg/L, a 50% dose reduction was suggested in **Regimen 1**. In comparison, both fixed dose amount reduction (3g or 4g) and 50% reduction were evaluated in the newly designed regimens (**Regimen 2 to 9)**. If a reduction resulted in a dose level lower than 0g, then 0g was applied. Besides, an additional concentration threshold of dose reduction, 18 mg/L, with 1g dose reduction was introduced in **Regimen 2 to 9,** since a 7-day period of no dose adjustment presented.

Reference

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2. Kerkhofs, T.M. *et al.* Comparison of Two Mitotane Starting Dose Regimens in Patients With Advanced Adrenocortical Carcinoma. *The Journal of Clinical Endocrinology & Metabolism* **98**, 4759-67 (2013).

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4. Abrantes, J.A., Jönsson, S., Karlsson, M.O. & Nielsen, E.I. Handling interoccasion variability in model‐based dose individualization using therapeutic drug monitoring data. *British journal of clinical pharmacology* **85**, 1326-36 (2019).

Table S1 Potential SNPs out of the 959 SNPs that are correlated to mitotane clearance based on the association analysis

	Gene	Common Name	dbSNP.RS.ID	P value
1	*CYP2C18*	CYP2C18_c.1154C>T(T385M)	rs2281891	0.020
2	*CYP2C19*	CYP2C19*2_19154G>A(P227P)	rs4244285	0.020
3	*SLCO1B3*	SLCO1B3_c.334G>T(A112S)	rs4149117	0.027
4	*SLCO1B3*	SLCO1B3_c.699A>G(I233M)	rs7311358	0.027
5	*SLCO1B3*	SLCO1B3_c.1557G>A(A519A)	rs2053098	0.027
6	*SLCO1B1*	SLCO1B1_c.571T>C(L191L)	rs4149057	0.020
7	*VKORC1*	VKORC1_c.*134G>A(3'UTR)	rs7294	0.050
8	*VKORC1*	VKORC1_c.283+124G>C	rs8050894	0.030
9	*VKORC1*	VKORC1_c.174-136C>T	rs9934438	0.030
10	*VKORC1*	VKORC1_c.-1639G>A(Promoter)	rs9923231	0.030
11	*UGT1A6*	UGT1A6_c.315A>G(L105L)	rs1105880	0.042

Table S2 Additional potential SNPs that are correlated to mitotane clearance based on the association analysis, if the pre-set selection based on a translation file as recommended by Affymetrics® was not considered.

	Gene	Common Name	dbSNP.RS.ID	P value
1	*CA5P*	CA5P_A>G(rs11859842)	rs11859842	0.029
2	*SLC16A1*	SLC16A1_c.*1942T>C	rs9429505	0.0067
3	*CHST10*	CHST10_c.*381G>A	rs1530031	0.040
4	*CYP20A1*	CYP20A1_50767C>T(L346F)	rs1048013	0.014
5	*SLC22A13*	SLC22A13_c.*8336G>A	rs4679028	0.032
6	*UGT2A1*	UGT2A1_c.1305-109A>C	rs2288741	0.042
7	*ADH6*	ADH6_c.-930T>C	rs10002894	0.012
8	*ADH6*	ADH6_c.-2874T>C	rs6830685	0.012
9	*SLCO5A1*	SLCO5A1_c.97C>T(L33F)	rs3750266	0.015

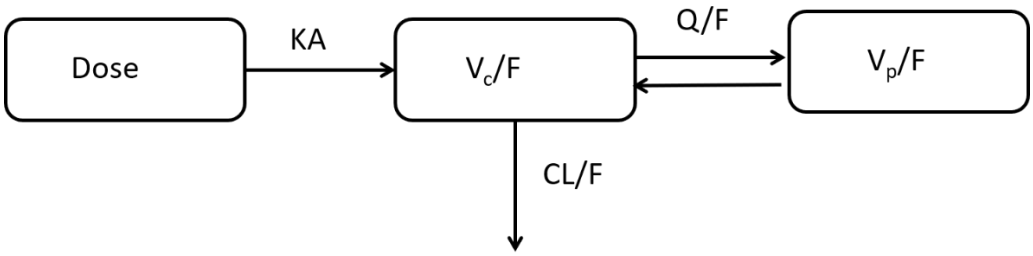

Fig. S1. The population PK model structure of mitotane. CL/F represents apparent system clearance, KA represents absorption rate constant, Vc/F represents apparent distribution volume of central compartment, Vp/F represents apparent distribution volume of peripheral compartment, Q/F represents apparent distribution rate constant.

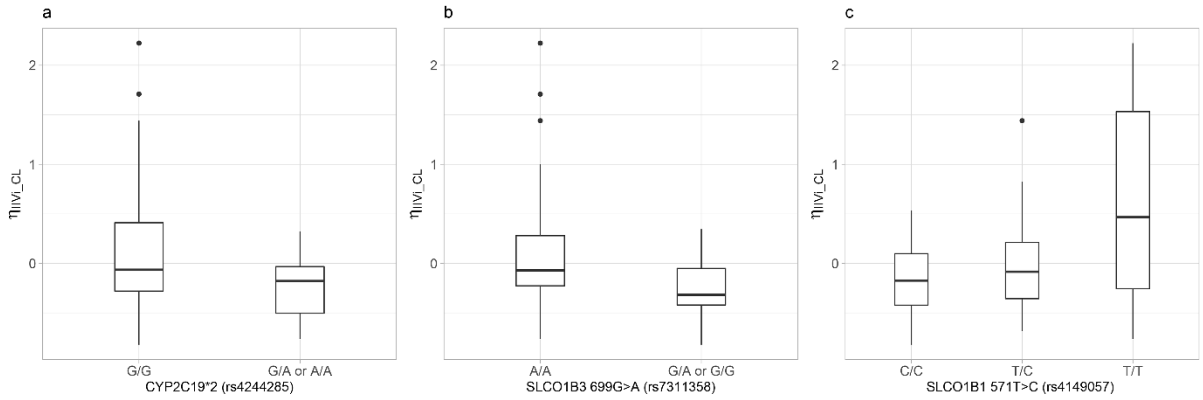

Fig. S2 The boxplots of estimated η_{IIVi_CL} in each genotype group of SNP (a) CYP2C19*2 (rs4244285), (b) SLCO1B3 699A>G (rs7311358), and (c) SLCO1B1 571T>C (rs4149057)

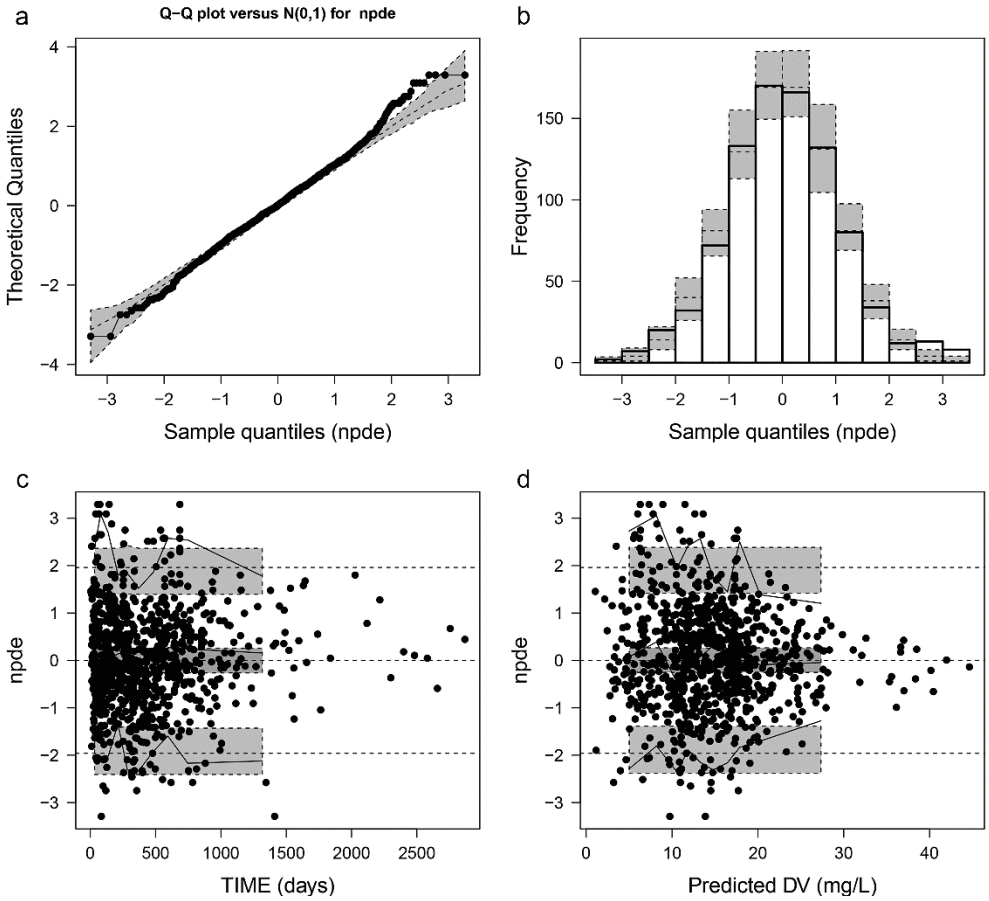

Fig. S3. Normalized prediction distribution error (NPDE) results of the final population PK model of mitotane in patients with ACC, including the quantile–quantile plot (a), the distribution histogram of NPDE (b), and the NPDE versus time (c) and population predictions (d). The NPDE results are shown to distribute around a mean of 0.03616 with a variance of 1.134.

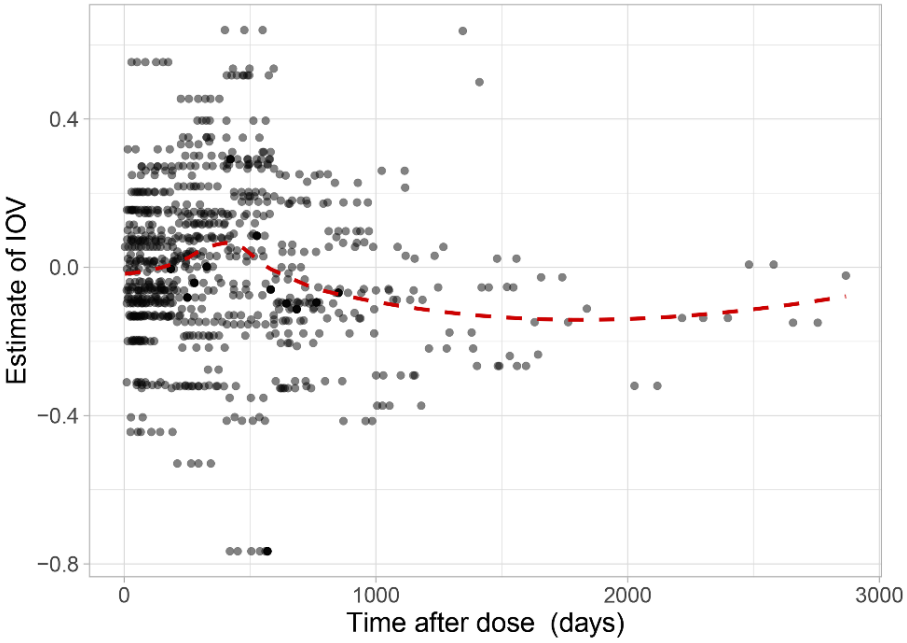

Fig. S4. The estimates of inter-occasion variability (IOV) over time. Red dashed lines represent loess regression result.

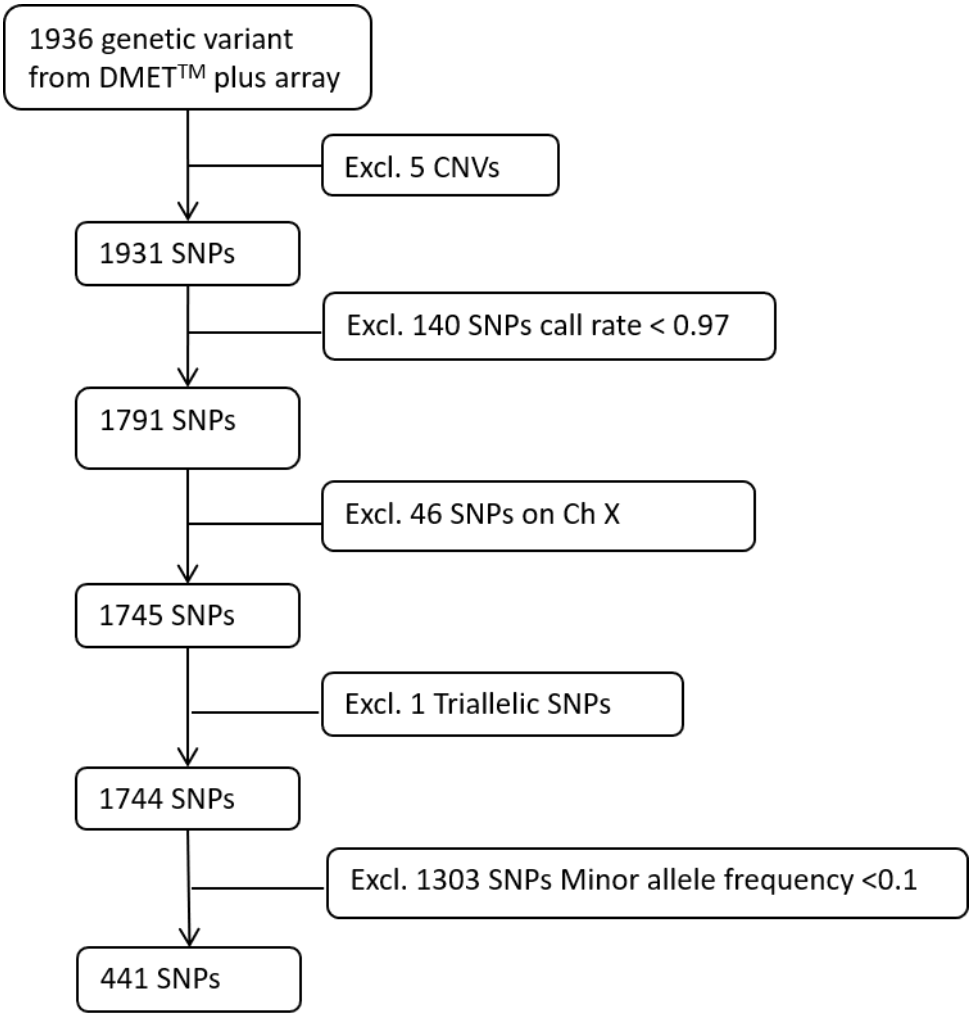

Fig. S5 Flow diagram of the genetic variants selection if the pre-set selection based on a translation file as recommended by Affymetrics® was not considered. Excl. represents excluding, Ch X represents chromosome X, DMET™ represent Drug Metabolizing Enzymes and Transporters, CNVs represents copy number variations.