UGT1A3 and Sex Are Major Determinants of Telmisartan Pharmacokinetics—A Comprehensive Pharmacogenomic Study

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To investigate how variability in multiple pharmacokinetic genes associates with telmisartan exposure, we determined telmisartan single-dose (40 mg) pharmacokinetics and sequenced 379 genes in 188 healthy volunteers. Intronic UGT1A variants showed the strongest associations with the area under the plasma concentration-time curve from zero hours to infinity (AUC0–∞) and peak plasma concentration (Cmax) of telmisartan. These variants were strongly linked with the increased function UGT1A3*2 allele, suggesting that it is the causative allele underlying these associations. In addition, telmisartan plasma concentrations were lower in men than in women. The UGT1A3*2 was associated with a 64% and 63% reduced AUC0-∞ of telmisartan in UGT1A3*2 heterozygous and homozygous men, respectively (P = 1.21 × 10−16 and 5.21 × 10−8). In women, UGT1A3*2 heterozygosity and homozygosity were associated with 57% (P = 1.54 × 10−11) and 72% (P = 3.31 × 10−15) reduced AUC0-∞, respectively. Furthermore, a candidate gene analysis suggested an association of UGT1A3*3 and the SLCO1B3 c.767G>C missense variant with telmisartan pharmacokinetics. A genotype score, which reflects the effects of sex and genetic variants on telmisartan AUC0–∞, associated with the effect of telmisartan on diastolic blood pressure. These data indicate that sex and UGT1A3 are major determinants and suggest a role for OATP1B3 in telmisartan pharmacokinetics.

Telmisartan is an angiotensin II receptor antagonist for treatment of hypertension.1 Uridine diphosphate-glucuronosyltransferase (UGT) 1A3 metabolizes telmisartan extensively to an acyl-glucuronide metabolite, which is the only telmisartan metabolite found in humans.2,3 UGT1A1, 1A7, 1A8, and 1A9 may also contribute to telmisartan glucuronidation.3 Furthermore, in vitro studies have suggested the involvement of OATP1B3 (encoded by SLCO1B3) and 2B1 (encoded by SLCO2B1) transporters in the cellular uptake and P-gp/MDR1 (encoded by ABCB1) in the cellular efflux of telmisartan.4–7 Telmisartan acyl-glucuronide is a substrate of OATP1B3 and it is eliminated mainly by biliary excretion.2,8,9

High interindividual variability exists in the pharmacokinetics of telmisartan and low exposure to telmisartan is associated with poor blood pressure-lowering effect.10–12 Telmisartan is usually effective with a 40 mg daily dose, but a significant proportion of patients require a higher dose or combination treatment to achieve sufficient blood pressure control.13 For optimal therapy, it would be important to understand the sources of variability in the response to antihypertensive medication.

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There are few previous studies on the associations of genetic variants on the pharmacokinetics of telmisartan with relatively small sample sizes, but comprehensive studies are lacking. In two of the studies, either the UGT1A3*2 or the UGT1A1*28 allele was associated with significantly reduced plasma concentrations of telmisartan. In addition, one small study suggested an association associated with significantly reduced plasma concentrations of telmisartan peak plasma concentration (Cmax). On the other hand, variants in, for example, CYP2C8, CYP2C9, UGT2B7, SLC01B3, ABCB1, and ABCG2 have not been associated with telmisartan pharmacokinetics. The aim of this study was to evaluate how variations in 379 pharmacokinetic genes associate with telmisartan pharmacokinetics.

METHODS
A total of 188 healthy unrelated white Finnish volunteers participated in the study after giving written informed consent. Their health was confirmed by medical history, clinical examination, and laboratory tests. Participants were not on any continuous medication nor were tobacco smokers. The study was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (record number 267/13/03/00/2011) and the Finnish Medicines Agency Fimea (EudraCT number 2011-00465-40). Of the participants, 91 were women and 97 men. Their mean ± SD age was 24 ± 4 years, height 174 ± 9 cm, body weight 69 ± 12 kg, and body mass index 22.8 ± 2.6 kg/m².

Telmisartan pharmacokinetics and pharmacodynamics
After an overnight fast, each participant ingested a 40 mg dose of telmisartan (Micardis tablet; Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany) with 150 mL of water at 8 AM. Standardized meals were served at 4, 7, and 10 hours after telmisartan ingestion. Timed blood samples (4–9 mL each) were collected to light-protected EDTA tubes prior to and 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 12, 24, and 48 hours after telmisartan administration. Tubes were immediately placed on ice. Plasma was separated within 30 minutes and stored at −70°C until analysis. Systolic and diastolic blood pressures were measured in a sitting position with an automatic oscillometric blood pressure monitor (Omron Healthcare Europe BV, Hoofddorp, The Netherlands) before and at 4, 12, and 24 hours after telmisartan ingestion. The pharmacodynamic variables, the average change in diastolic and systolic blood pressure, were calculated by dividing the incremental area under the blood pressure-time curve from time 0 to 24 hours with 24 hours.

Telmisartan and telmisartan acyl-(β-D)-glucuronide plasma concentrations were quantified using a Nexera X2 ultra-high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) coupled to a Qtrap 5500 mass spectrometer (ABSciex, Foster City, CA). Plasma samples were pretreated using a Phree phospholipid removal plate (Phenomenex, Torrance, CA) according to the manufacturer’s instructions. In brief, plasma and acetonitrile containing the internal standards were mixed in a ratio of 1:5 (v/v), and the mixture was allowed to incubate for 10 minutes at room temperature. The sample mixture was then filtered through the cartridge, diluted with mobile phase (A), and delivered to the ultra-high-performance liquid chromatography system. Telmisartan and telmisartan acyl-glucuronide were separated on a Kinetex Biphenyl (2.6 µm, 2.1 mm × 100 mm; Phenomenex) using a gradient elution. Mobile phase consisted of 10 mM ammonium formate (pH 3.2, adjusted with formic acid) (A) and acetonitrile (B). The mobile phase gradient was a linear ramp from 30% B to 97% B over 1 minute, followed by 0.5 minutes at 97% B on hold, and an equilibration step back to the starting composition. The flow rate and the column temperature were maintained at 350 µL/min and 30°C, and the injection volume was 5 µL. Isotope-labeled analogs served as internal standards for both analytes. The mass spectrometer was operated in a positive polarity mode and the targeted mass-to-charge ratio (m/z) ion transitions were 515–276 and 691–515 for telmisartan and telmisartan acyl-glucuronide. The lower limits of quantification were 0.3 ng/mL and 0.1 ng/mL, respectively. The day-to-day precision values (coefficients of variation) for both compounds were below 15% and accuracy within ±15%, except for the lower limits of quantification, for which both precision and accuracy were within 20%.

The areas under the plasma concentration-time curve from 0 hours to infinity (AUC0–∞), Cmax, and the elimination half-life (t1/2) values were calculated for telmisartan and telmisartan acyl-glucuronide with standard noncompartmental methods using Phoenix WinNonlin, version 6.3 (Certara, Princeton, NJ).

DNA sequencing and genotyping
Genomic DNA was extracted from EDTA blood samples using the Maxwell 16 LEV Blood DNA Kit on a Maxwell 16 Research automated nucleic acid extraction system (Promega, Madison, WI). A total of 379 pharmacokinetic genes, comprising phase I and II metabolizing enzymes, influx and efflux drug transporters, and regulatory proteins, were selected to be studied. These genes ≥20, were completely sequenced in the study participants using targeted massive parallel sequencing at the Technology Centre at Institute for Molecular Medicine Finland (Helsinki, Finland). NEBNext DNA Sample Prep protocol (New England BioLabs, Ipswich, MA) was used for library preparation and the NimbleGen SeqCap EZ Choice protocol (Roche Sequencing, Pleasanton, CA) for target enrichment capture. Sequencing was done on the Illumina HiSeq2000 platform with 100 bp paired-end reads (llumina, San Diego, CA). Quality control, short read alignment, and variant calling and annotation were carried out using an in-house developed pipeline. Mean coverage depth was 37.2X. Coverage depth ≥10X, Hardy–Weinberg equilibrium P < 3.15 × 10⁻⁷ (Bonferroni correction), and proportion missing ≤0.05 were used as quality thresholds. A total of 46,064 SNVs with minor allele frequency (MAF) ≥0.05 passed these criteria and were included in the statistical analysis. In order to verify genotype calls and to supplement missing data, all study participants were genotyped with TaqMan genotyping assays on a QuantStudio 12K Flex Real-Time PCR System for the UGT1A rs13401281, rs3821242, rs4663969, rs6715325, rs6431625, rs45449995, rs45148323, and rs3064744 sequence variations (Thermo Fisher Scientific, Waltham, MA). Call identity with sequencing data was 99.5% for rs6715325 and 100% for all other SNVs. In case of discordant results, genotypes obtained by sequencing were used in the statistical analysis.

Statistical analysis
The number of participants was estimated to be sufficient to detect an effect size of R² larger than 0.2 with two predictors in multiple linear regression analysis, with a power > 80% (Bonferroni corrected or level 1.09 × 10⁻⁶). The data were analyzed with the statistical programs JMP Genomics 8.2 (SAS Institute, Cary, NC) and IBM SPSS 22.0 for Windows (Armonk, NY). The pharmacokinetic variables were logarithmically transformed before analysis. Sex and body weight were tested as demographic covariates for pharmacokinetic data using stepwise linear regression analysis, with P value thresholds of 0.05 for entry and 0.10 for removal. Possible associations of genetic variants with pharmacokinetic variables were investigated using linear regression analysis fixed for significant demographic covariates with a stepwise approach. A Bonferroni corrected P value threshold of 1.09 × 10⁻⁶ was used for the 379 gene and thresholds of 0.05 for entry and 0.10 for removal for the candidate gene analysis. Additive coding was used for genetic variants in the 379 gene analysis and both additive and dummy variable coding in the candidate gene analysis. HaploPyte simulations for UGT1A gene were performed with PHASE version 2.1.1. Possible comparisons of proportions were done using the Fisher’s exact test.
AUC with the change in blood pressure during 24 hours was investigated using partial correlation analysis controlling for baseline blood pressure. Comparison of the change in blood pressure during 24 hours between genotype scores was investigated with analysis of variance, with baseline blood pressure as a covariate and P value < 0.05 considered statistically significant. Pharmacokinetic data are given as geometric means with geometric coefficients of variation and ranges, geometric SDs, or 90% confidence intervals (CIs). Pharmacodynamic data are given as arithmetic means with 95% CIs and proportions with 95% CIs.

RESULTS

Telmisartan pharmacogenomics

Among the 188 healthy volunteers, the AUC$_{0–\infty}$ and C$_{\text{max}}$ of telmisartan varied 49-fold and 31-fold, respectively, and those of telmisartan acyl-glucuronide 18-fold and 17-fold, respectively (Table 1). Both telmisartan AUC$_{0–\infty}$ and C$_{\text{max}}$ were larger in women than in men. In addition, body weight was a significant covariate for telmisartan C$_{\text{max}}$. When entering both sex and body weight as independent variables in the regression analysis for telmisartan AUC$_{0–\infty}$, sex (P = 0.005) but not body weight (P = 0.405) remained significantly associated. This indicates that the association of sex is independent of the weight.

In a stepwise linear regression analysis, which tested the associations of 46,064 SNVs with MAF of at least 0.05, the rs6715325 SNV, located between the UGT1A3 and UGT1A4 first exons, showed the strongest association with the AUC$_{0–\infty}$ of telmisartan (Table 2, Figure 1). The AUC$_{0–\infty}$ was 46% (P = 1.81 × 10$^{-22}$) smaller per copy of the variant allele. Furthermore, the rs2361501 SNV, located after UGT1A3 first exon, showed the strongest association with telmisartan C$_{\text{max}}$, which was 26% (P = 4.00 × 10$^{-7}$) smaller per copy of the variant allele. After adjusting for the rs6715325 or rs2361501 variant, no other variants remained associated with telmisartan AUC$_{0–\infty}$ or C$_{\text{max}}$ at the Bonferroni corrected significance level. The investigated genetic variants were not significantly associated with the t$_{1/2}$ of telmisartan.

Similar to parent telmisartan, telmisartan acyl-glucuronide C$_{\text{max}}$ was significantly associated with intronic SNVs located around UGT1A3 first exon. The strongest association was observed with four SNVs (rs13401281, rs11891311, rs7564935, and rs11888459) in complete linkage disequilibrium with each other. The C$_{\text{max}}$ was 39% (P = 6.25 × 10$^{-11}$) higher per copy of the variant allele (Table 2). After adjusting for any one of these variants, no other variants remained statistically significantly associated with the C$_{\text{max}}$. The investigated variants were not significantly associated with the AUC$_{0–\infty}$ or t$_{1/2}$ of telmisartan acyl-glucuronide. The telmisartan acyl-glucuronide/telmisartan AUC$_{0–\infty}$ ratio also showed the strongest association with a UGT1A variant (Table 2).

Linkage disequilibrium and haplotype analysis

In order to identify the causative SNVs underlying the association of the top noncoding UGT1A SNVs (rs6715325, rs2361501, rs13401281, and rs4663969) with telmisartan pharmacokinetic variables, we next investigated the linkage disequilibrium profile and computed haplotypes across the UGT1A gene. This was done for the top noncoding SNVs and the missense and functional variants (MAF ≥ 0.01) of the UGT1As, which metabolize telmisartan in vitro (UGT1A1, UGT1A3, UGT1A7, and UGT1A9; Figure 2). All the top noncoding SNVs were strongly linked with each other as well as with the two UGT1A3 c.31T>C (p.Trp11Arg, rs3821242) and c.140T>C (p.Val47Ala, rs6431625) missense SNVs, which define the UGT1A3*2 allele (Figure 2a). The top SNVs were also in a relatively strong linkage disequilibrium with the UGT1A4*28 allele (rs3064744, c.5475insTA, TA6>TA7) and with the UGT1A7 c.622T>C (p.Trp208Arg, rs11692021) missense SNV.

Altogether, 25 haplotypes were inferred in the haplotype computation (Figure 2b). The top noncoding SNVs were present in

Table 1 Pharmacokinetic variables of telmisartan and telmisartan acyl-glucuronide in 188 healthy volunteers and the effects of significant demographic covariates on these variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Geometric mean</th>
<th>CV</th>
<th>Range</th>
<th>Demographic covariate</th>
<th>Effect (90% CI)$^{a}$</th>
<th>P value</th>
<th>Adjusted R$^{2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Telmisartan</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C$_{\text{max}}$, ng/mL</td>
<td>35</td>
<td>80%</td>
<td>5.2–165</td>
<td>Body weight</td>
<td>−14.2% (−18.7%, −9.5%)</td>
<td>4.01 × 10$^{-6}$</td>
<td>0.0150</td>
</tr>
<tr>
<td>T$_{\text{max}}$, hour$^{b}$</td>
<td>1.5</td>
<td>—</td>
<td>0.5–9</td>
<td>Sex</td>
<td>31.9% (9.5%, 59.0%)</td>
<td>0.0150</td>
<td>0.27</td>
</tr>
<tr>
<td>AUC$_{0–\infty}$, ng·hour/mL</td>
<td>308</td>
<td>84%</td>
<td>37–1797</td>
<td>Sex</td>
<td>55.9% (31.7%, 84.6%)</td>
<td>2.26 × 10$^{-5}$</td>
<td>0.09</td>
</tr>
<tr>
<td>t$_{1/2}$, hour</td>
<td>18</td>
<td>41%</td>
<td>6.7–83</td>
<td>Body weight</td>
<td>−4.2% (−6.8%, −1.6%)</td>
<td>9.41 × 10$^{-3}$</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Telmisartan acyl-glucuronide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_{\text{max}}$, ng/mL</td>
<td>4.4</td>
<td>58%</td>
<td>1.2–19.4</td>
<td>Body weight</td>
<td>−10.7% (−13.8%, −7.5%)</td>
<td>2.54 × 10$^{-7}$</td>
<td>0.13</td>
</tr>
<tr>
<td>T$_{\text{max}}$, hour$^{b}$</td>
<td>0.75</td>
<td>—</td>
<td>0.5–12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>AUC$_{0–\infty}$, ng·hour/mL</td>
<td>74</td>
<td>50%</td>
<td>18–318</td>
<td>Body weight</td>
<td>−7.7% (−10.5%, −4.7%)</td>
<td>3.76 × 10$^{-5}$</td>
<td>0.08</td>
</tr>
<tr>
<td>t$_{1/2}$, hour</td>
<td>20</td>
<td>58%</td>
<td>7.9–94</td>
<td>Sex</td>
<td>18.5% (4.3%, 34.7%)</td>
<td>0.0296</td>
<td>0.02</td>
</tr>
<tr>
<td>Telmisartan acyl glucuronide/ telmisartan AUC$_{0–\infty}$ ratio</td>
<td>0.24</td>
<td>73%</td>
<td>0.05–1.4</td>
<td>Sex</td>
<td>−20.1% (−31.7%, −6.7%)</td>
<td>0.018</td>
<td>0.03</td>
</tr>
</tbody>
</table>

AUC$_{0–\infty}$, area under the plasma concentration-time curve from 0 hour to infinity; CI, confidence interval; CV, geometric coefficient of variation; C$_{\text{max}}$, peak plasma concentration; T$_{\text{max}}$, concentration peak time; t$_{1/2}$, elimination half-life.

$^{a}$Per 10% increase in body weight; sex: women vs. men. $^{b}$T$_{\text{max}}$ data given as median.
Table 2 Results of the stepwise forward linear regression analysis of the effects of 46,064 SNVs in 379 genes on telmisartan pharmacokinetics

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pharmacokinetic variable</th>
<th>Nucleotide change</th>
<th>Location</th>
<th>Gene</th>
<th>dbSNP ID</th>
<th>Minor allele frequency (MAF)</th>
<th>Adjusted value (P)</th>
<th>90% CI</th>
<th>dbSNP ID</th>
<th>Minor allele frequency (MAF)</th>
<th>Adjusted value (P)</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Telmisartan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.81 × 10^{-22}</td>
<td>0.46</td>
<td>rs6715325</td>
<td>0.46</td>
<td>65.6%</td>
<td>50.3%, 70.5%</td>
</tr>
<tr>
<td></td>
<td>Telmisartan acyl-glucuronide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.25 × 10^{-11}</td>
<td>0.40</td>
<td>rs3401281</td>
<td>0.40</td>
<td>39.2%</td>
<td>28.6%, 50.6%</td>
</tr>
</tbody>
</table>

Candidate gene analysis

Due to the risk of false negative associations with the conservative Bonferroni correction in the primary analysis, we next carried out a candidate gene analysis for telmisartan AUC\(^{0\to\infty}\), without correction for multiple testing. In this analysis, we included missense and functional variants with MAF of ≥ 0.01 in genes suggested to be involved in telmisartan pharmacokinetics (UGT1A3, UGT1A1, UGT1A7, UGT1A8, UGT1A9, SLCO1B3, SLCO1B1, ABCB1, and ABC2; Table S1). In the analysis, UGT1A3*2 was associated with a 48% (P = 3.82 × 10^{-27}) and UGT1A3*3 with a 28% (P = 7.01 × 10^{-3}) reduced AUC\(^{0\to\infty}\) per copy of the allele, and SLCO1B3 c.767G>C (p.Gly256Ala, rs60149050) with a 22% (P = 0.0392) increased telmisartan AUC\(^{0\to\infty}\) per copy of the minor allele (Table 3). Because of linkage disequilibrium throughout the whole UGT1A locus, we repeated the candidate gene analysis using the inferred UGT1A8-1A9-1A7-1A3-1A1 haplotypes with MAF ≥ 0.01 (Figure 2b). All the haplotypes, which contained UGT1A3*2, were associated with reduced AUC\(^{0\to\infty}\) of telmisartan (Table S2).

Interestingly, the UGT1A3*2 allele lacked gene-dose effect especially in men and thus the log-additive regression model seemed to overestimate the AUC\(^{0\to\infty}\) of heterozygous UGT1A3*2 carriers. We, therefore, repeated the candidate gene analysis with UGT1A3*2 divided to four dummy variables representing the heterozygous and homozygous men and women. In this analysis, UGT1A3*2 heterozygosity and homozygosity in men were associated with a 64% (P = 1.21 × 10^{-16}) and 63% (P = 5.21 × 10^{-8}) reduced telmisartan AUC\(^{0\to\infty}\), respectively (Table 3). In women, the UGT1A3*2 heterozygosity and homozygosity were associated with 57% (P = 1.54 × 10^{-11}) and 72% (P = 3.31 × 10^{-15}) reduced AUC\(^{0\to\infty}\), respectively. Entering body weight as a covariate in addition to sex did not change the results.

To further investigate the roles of UGT variants in telmisartan glucuronidation, we next carried out a candidate gene analysis for telmisartan acyl-glucuronide/telmisartan AUC\(^{0\to\infty}\) ratio with UGT1A3*2 dummy variables and other UGT1A3 and UGT1A1, UGT1A7, UGT1A8, and UGT1A9 variants. UGT1A3*2 heterozygosity and homozygosity in men were associated with a 177% (P = 3.13 × 10^{-27}) and 293% (P = 2.25 × 10^{-18}) increased telmisartan acyl-glucuronide/telmisartan AUC\(^{0\to\infty}\) ratio, respectively (Table S3). In women, the UGT1A3*2 heterozygosity and homozygosity were associated with 116% (P = 8.59 × 10^{-16}) and 250% (P = 1.19 × 10^{-21}) increased telmisartan acyl-glucuronide/telmisartan AUC\(^{0\to\infty}\) ratio, respectively.

To predict telmisartan AUC\(^{0\to\infty}\) in men and women with different combinations of UGT1A3 and SLCO1B3 genotypes, we calculated genotype scores (GS) using the UGT1A3*2 dummy haplotypes which contained UGT1A3*2 or UGT1A3*3. In addition, the rs2361501 SNV was present in the same haplotype with UGT1A3*6. Altogether, the UGT1A3*2 allele was present in 10, UGT1A3*3 in 4, and UGT1A3*6 in 1 of the 25 inferred haplotypes. The sum of frequencies was 0.39 for the UGT1A3*2, 0.061 for UGT1A3*3, and 0.019 for the UGT1A3*6 containing haplotypes. The most frequent haplotype (n = 98; MAF 0.26) contained the UGT1A3*2, UGT1A7*3, and UGT1A1*28 alleles.
variable candidate gene linear regression model with the following equation:

$$G_{\text{telmisartan}} = 1.47^{(M)} + 1^{(F)} \times UGT1A3 \times 2 \times 0.74^{(UGT1A3*3)} \times 1.23^{(SLCO1B3 c.767G>C)}$$

Where M is male and F is female, $UGT1A3*2$ factor is 0.36 for heterozygous men, 0.37 for homozygous men, 0.42 for heterozygous women, 0.28 for homozygous women, and 1 for noncarriers of $UGT1A3*2$, and $n$ is the number of variant alleles (0, 1, or 2) of $UGT1A3*3$ and $SLCO1B3 c.767G>C$ (Figures 3 and 4). The GS is 1.00 in men who do not carry any of the variants. For others, the score shows the fold difference in telmisartan AUC$_{0-\infty}$ compared with 1.00.

**Pharmacodynamics**

A total of 65% and 23% of individuals with GS of below 0.5 and between 0.5 and 0.8 had AUC$_{0-\infty}$ values below 229 ng·hour/mL, which was found in a previous study to be the AUC$_{0-\infty}$ value giving 50% of telmisartan maximum effect (Figure 3). The respective percentages in individuals with GS between 0.8 and 1.25, and above 1.25 were 6% and 3%. Diastolic blood pressure decreased 1.3 mmHg (CI 95%, 0.05, 2.5 mmHg) less in individuals
Figure 2 (a) Linkage disequilibrium of the top noncoding SNVs and missense and functional variants of the UGT1As, which metabolize telmisartan in vitro. (b) UGT1A haplotypes inferred with missense and functional variants, and top noncoding UGT1A SNVs. Intronic variations are depicted in blue and missense variations in red. The * alleles are defined on the basis of missense variations within each UGT1A enzyme. The UGT1A7 allele containing the c.352G>T (p.Asp118Tyr, rs140814031) SNV together with c.386T>G, c.392G>A, and c.622T>C SNVs has not been named before and is here given tentatively the name UGT1A7*15. For clarity, only rs13401281 of the four completely linked telmisartan acyl-glucuronide peak plasma concentration (C_{max}) top SNVs (rs13401281, rs11891311, rs7564935, and rs11888459) is presented in this figure. MAF, minor allele frequency; SNV, single-nucleotide variation.
with GS below 0.8 than in those with GS above 0.8 ($P = 0.042$; Figure 3). The average change in systolic blood pressure was not significantly associated with the GS (data not shown). Telmisartan AUC$_{0-\infty}$ correlated negatively with the average change in diastolic blood pressure ($r = -0.16; P = 0.034$) and systolic blood pressures ($r = -0.19; P = 0.008$).

![Table 3 Results of the candidate gene analyses on telmisartan AUC$_{0-\infty}$](image)

### Table 3 Results of the candidate gene analyses on telmisartan AUC$_{0-\infty}$

<table>
<thead>
<tr>
<th>Pharmacokinetic variable</th>
<th>Covariate/SNV</th>
<th>Average (%)</th>
<th>90% CI</th>
<th>$P$ value</th>
<th>Adjusted $R^2$ for each step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan AUC$_{0-\infty}$ (Log-additive model)</td>
<td>UGT1A3*2</td>
<td>−48.5</td>
<td>−53.2, −43.2</td>
<td>$3.82 \times 10^{-22}$</td>
<td>0.447</td>
</tr>
<tr>
<td>Telmisartan AUC$_{0-\infty}$ (UGT1A3*2 dummy variables)</td>
<td>UGT1A3*2 homozygous men</td>
<td>−63.6</td>
<td>−69.6, −56.3</td>
<td>$1.21 \times 10^{-16}$</td>
<td>0.227</td>
</tr>
<tr>
<td>Telmisartan AUC$_{0-\infty}$ (UGT1A3*2 dummy variables)</td>
<td>UGT1A3*2 homozygous women</td>
<td>−71.6</td>
<td>−77.7, −63.9</td>
<td>$3.31 \times 10^{-15}$</td>
<td>0.321</td>
</tr>
<tr>
<td>Telmisartan AUC$_{0-\infty}$ (UGT1A3*2 dummy variables)</td>
<td>UGT1A3*2 heterozygous women</td>
<td>−57.3</td>
<td>−64.9, −48.1</td>
<td>$1.54 \times 10^{-11}$</td>
<td>0.439</td>
</tr>
<tr>
<td>Telmisartan AUC$_{0-\infty}$ (UGT1A3*2 dummy variables)</td>
<td>UGT1A3*2 homozygous men</td>
<td>−63.2</td>
<td>−72.5, −50.8</td>
<td>$5.21 \times 10^{-8}$</td>
<td>0.505</td>
</tr>
</tbody>
</table>

Figure 3 (a) The geometric mean ± geometric SD area under the plasma telmisartan concentration-time curve from zero hour to infinity (AUC$_{0-\infty}$) values grouped by genotype scores (GS). The UGT1A3*1 allele includes all other UGT1A3 alleles than UGT1A3*2 and *3. (b) GS for individuals with different genotype combinations. UGT1A3 and SLCO1B3 reference genotypes are depicted with white, heterozygous with gray, and homozygous variant genotypes with black rectangles. The estimated population frequencies of the genotype combinations were obtained from the 1000 Genomes Project Data. $^{18,48}$ (c) The percentage of individuals (95% confidence interval (CI)) with AUC$_{0-\infty} < 229$ ng·hour/mL in different GS groups. (d) The average change (95% CI) in diastolic blood pressure during 24 hours after telmisartan administration in GS groups < 0.8 and ≥ 0.8.
DISCUSSION
In this study, we investigated the associations of variations in 379 genes with telmisartan pharmacokinetics in 188 healthy volunteers. Noncoding variants in the UGT1A gene showed the strongest associations with telmisartan pharmacokinetics. These top variants were strongly linked with the UGT1A3*2 and UGT1A3*3 alleles, which provides a plausible mechanism for the association. Both UGT1A3*2 and UGT1A3*3 were associated with reduced systemic exposure to telmisartan. In addition, a missense variant in SLCO1B3 was associated with increased telmisartan exposure. Moreover, telmisartan exposure was significantly lower in men than in women. Based on these results, we constructed a scoring system to predict telmisartan plasma exposure in men and women with different combinations of genetic variants.

In this study, the UGT1A3*2 allele was associated with a markedly reduced telmisartan AUC and explained more than 40% of its interindividual variability. These results are in line with previous studies showing associations between UGT1A3*2 or linked UGT1A variants, such as UGT1A1*28, and reduced telmisartan exposure, and indicate that metabolism via UGT1A3 is the main route of telmisartan elimination. In human liver samples, the UGT1A3*2 allele has been associated with increased mRNA and protein expression of UGT1A3. In addition to telmisartan, the UGT1A3*2 allele has been associated with increased metabolism of other UGT1A3 substrates, namely montelukast, atorvastatin, and febuxostat. The causal variant in this haplotype has not yet been identified, however. The haplotype carrying UGT1A3*2 harbors many noncoding SNVs located around the first exon of UGT1A3, which might, for example, influence UGT1A3 mRNA transcription. Another explanation could be induction of UGT1A3 expression by bilirubin, whose intrahepatic concentration should be increased in individuals carrying the UGT1A3*2-linked UGT1A1*28.

In the candidate gene analysis, also the UGT1A3*3 allele was associated with decreased telmisartan AUC. The effect of this allele was, however, smaller than that of UGT1A3*2 and explained only 1–2% of the interindividual variability in telmisartan exposure. In previous studies with relatively small numbers of UGT1A3*3 carriers, the allele has not affected UGT1A3 mRNA or protein expression in human liver samples. In vitro studies on the effects of UGT1A3*3 on glucuronidation activity have been inconsistent, with studies showing either reduced or unchanged enzyme activity. Given the low allele frequency of UGT1A3*3 and the relatively weak association with telmisartan exposure, the result needs confirmation in future studies.

In accordance with the associations with parent telmisartan, the UGT1A3*2 allele was associated with increased telmisartan acyl-glucuronide $C_{\text{max}}$ and telmisartan acyl-glucuronide/telmisartan AUC ratio. In theory, the increased metabolite/parent compound AUC ratio could be explained by either increased formation or reduced elimination of the metabolite, or both. Given that the UGT1A3*2 allele was associated with a reduced AUC and thus an increased oral clearance of telmisartan, increased metabolite formation is the most probable explanation. The increased $C_{\text{max}}$ of telmisartan acyl-glucuronide in association with the UGT1A3*2 allele is likely explained by formation of the metabolite during the first pass. Despite a strong association with the AUC of parent telmisartan, the UGT1A3*2 allele was not associated with the AUC of the acyl-glucuronide metabolite. This indicates that UGT1A3*2 increases the rate of glucuronidation of telmisartan, but that the total amount of glucuronide formed is not changed. This is consistent with glucuronidation being the exclusive route of telmisartan elimination.

In addition to UGT1A3, which metabolizes telmisartan with the highest affinity in vitro, telmisartan is a substrate of UGT1A1, UGT1A7, UGT1A8, and UGT1A9. All UGT1A enzymes are encoded by the UGT1A gene in chromosome 2. They share exons 2–5, but have unique first exons. Strong linkage disequilibrium patterns span the entire UGT1A gene. In the present study, we fully sequenced the whole UGT1A gene. Of the variants in the other candidate UGT1As, the UGT1A1*28 and UGT1A7*3 alleles are strongly linked with UGT1A3*2. The UGT1A1*28 allele has been associated with decreased glucuronidation of UGT1A1 substrates due to significantly decreased UGT1A1 protein expression. Moreover, the UGT1A7*3 allele has been associated with decreased function of UGT1A7 in vitro. After adjusting for the UGT1A3 effect, no other UGT1A variants, including UGT1A1*28 and UGT1A7*3, were significantly associated with telmisartan AUC. Similarly, in the haplotype-based analysis, the UGT1A1*28 and UGT1A7*3 alleles did not associate independently with telmisartan pharmacokinetics (Table S2). Taken together, these data indicate that genetic variability in UGT1A enzymes other than UGT1A3 is not of major importance for telmisartan pharmacokinetics.

In this study, the UGT1A3*2 allele seemed to lack a clear gene-dose effect on telmisartan AUC and $C_{\text{max}}$. That is, men showed no difference and women showed only a relatively small difference between UGT1A3*2 homozygotes and heterozygotes. No obvious explanation exists for these findings. However, linkage between
**UGT1A3**

**UGT1A3** and **UGT1A1** and a role of **UGT1A1** in intestinal telmisartan metabolism may play a role. It is notable, that the effects of the **UGT1A1** allele are generally much more pronounced in homozygotes than in heterozygotes, whereas those of **UGT1A3** are usually seen already in heterozygotes. In theory, the effects of **UGT1A1** might thus counteract the effects of **UGT1A3** on telmisartan pharmacokinetics in **UGT1A3**/2−**UGT1A3**/28 homozygotes.

Considering that telmisartan pharmacokinetics strongly depends on **UGT1A3** activity, it could be susceptible to **UGT1A3**-mediated drug-drug interactions. Interestingly, nifedipine has increased the AUC of telmisartan by 132%. In human liver microsomes, nifedipine inhibits the glucuronidation of estradiol, mediated by **UGT1A1**, **UGT1A3**, **UGT1A8**, **UGT1A10**, and **UGT2B7**. Further studies are required to determine whether nifedipine inhibits **UGT1A3**. Telmisartan could be a useful index substrate for **UGT1A3**-mediated drug-drug interactions.

Telmisartan has been suggested to be a relatively selective substrate of **OATP1B3**, an influx transporter expressed on the basolateral membrane of hepatocytes. In the candidate gene analysis, the **SLCO1B3** c.767G>C missense SNV was associated with increased telmisartan AUC, with a 22% increase per minor allele copy. However, this SNV explained only 1% of the interindividual variability in telmisartan exposure. There seems to be no previous studies investigating the associations of this SNV with telmisartan or other OATP1B3 substrate pharmacokinetics in humans. The **SIFT** and **PolyPhen in silico** prediction tools suggest the c.767G>C SNV to be deleterious. In one **in vitro** study, however, this SNV did not significantly affect the uptake of the OATP1B3 substrate cholecystokinin-8. A recent **in vitro** study suggested that OATP2B1 would play a more important role than OATP1B3 in the hepatic uptake of telmisartan. In our study, SNVs in **SLCO2B1** were not, however, associated with telmisartan pharmacokinetics. The analysis included, for example, the c.601G>A SNV associated previously with 3S,5R-fluvastatin and the c.1457C>T SNV associated previously with 3S,5R-fluvastatin, fexofenadine, and celiprolol pharmacokinetics.

In accordance with previous studies, the AUC and C\(_{\text{max}}\) of telmisartan were significantly lower in men than in women. Body size did not explain this difference. Our finding that the telmisartan acyl-glucuronide/telmisartan AUC ratio was higher in men than in women suggests that the difference could be due to higher telmisartan glucuronidation capacity in men. Interestingly, no sex difference seemed to exist in telmisartan AUC and C\(_{\text{max}}\) in individuals homozygous for **UGT1A3**.

Telmisartan plasma concentration–time profiles are characterized by a biexponential decline after the C\(_{\text{max}}\) with a rapid initial distribution followed by a prolonged terminal elimination phase. Of note, no appreciable drug accumulation occurs during multiple dosing. This suggests that our pharmacokinetic results after a single telmisartan dose can be extrapolated to continuous treatment. However, advanced age, concomitant diseases, and other medications may increase variability in telmisartan pharmacokinetics in patients with hypertension as compared with the healthy volunteers of the present study. Nevertheless, the effects of **UGT1A1** variants seem to be similar in patients with hypertension and healthy volunteers. This suggests that the effects of genetic variants on telmisartan pharmacokinetics in healthy volunteers can be extrapolated to patients with hypertension.

The maximum antihypertensive effect of telmisartan is usually attained within 4–8 weeks after the start of treatment, but some effect can be seen already 3 hours after the first dose in patients with hypertension. In general, the antihypertensive effect of telmisartan is much more pronounced in hypertensive than in normotensive individuals. Nevertheless, in our study in normotensive healthy volunteers, the average change in diastolic and systolic blood pressures correlated negatively with telmisartan AUC. Moreover, a low GS was associated with the weak diastolic blood pressure-lowering effect of telmisartan. One should keep in mind, however, that these pharmacodynamic results should not be directly extrapolated to patients with hypertension. The finding that the average change in systolic blood pressure was not significantly associated with the GS may be explained by greater variation in systolic than diastolic blood pressure.

A previous angiotensin II challenge study in healthy volunteers showed that telmisartan efficacy is concentration-dependent. The telmisartan AUC\(_{0\rightarrow\infty}\) value giving 50% of the maximum effect was estimated to be 229 ng × hour/mL. In the present study, the proportion of individuals having AUC\(_{0\rightarrow\infty}\) values below 229 ng·hour/mL after a 40 mg dose increased along with a decreasing GS. In the treatment of hypertension, the effective dose of telmisartan is usually 40 mg once daily, but a significant proportion of patients require 80 mg daily due to poor efficacy. Because the effect of telmisartan is dose-dependent and concentration-dependent, and there is large variability in telmisartan exposure, it is probable that inadequate efficacy with the 40 mg dose may, in some cases, be due to insufficient plasma concentrations. On the other hand, although telmisartan is usually well-tolerated, it is possible that increased exposure predisposes to hypotension or other adverse effects. The GS might aid in finding the right telmisartan doses for patients with hypertension.

The participants of this study were white Finnish volunteers, among whom the allele frequency of **UGT1A3** was 0.39. The **UGT1A3** allele is generally very common in European (0.32), Sub-Saharan African (0.56), and South-Asian (0.42) populations, but less common in East Asians (0.12). On the contrary, **UGT1A3** is more common in South and East Asians (MAF 0.20 and 0.18) as compared with Europeans (0.07) and Sub-Saharan Africans (0.086). These interethnic differences in **UGT1A3** allele frequencies lead to differences in the distributions of telmisartan GSs among populations (Figure 3). In previous studies in Asian individuals, the **UGT1A3** allele or linked **UGT1A** variants have been associated with a similar effect on telmisartan pharmacokinetics, as was seen in the present study. This suggests that our findings can be extrapolated to other populations.

In conclusion, these results indicate that genetic variants of **UGT1A3** associate strongly with the pharmacokinetics of telmisartan. Due to lower plasma concentrations, carriers of **UGT1A3** may be at an increased risk of poor blood pressure-lowering efficacy of telmisartan. The results also suggest an association of **SLCO1B3** c.767G>C variant with telmisartan
exposure. The generated GS may aid in individualizing treatment with telmisartan.

SUPPORTING INFORMATION
Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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CONFLICT OF INTEREST
All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
P.H. and M.Ni. wrote the manuscript. P.H., A.T., J.T.B., and M.Ni. designed the research. P.H., A.T., T.L., M.Ne., T.T., M.P.-H., J.T.B., and M.Ni. performed the research. P.H. and M.Ni. analyzed the data.

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