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Effect of ABCB1 haplotypes on the pharmacokinetics and renin-inhibiting effect of aliskiren

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Effect of *ABCB1* haplotypes on the pharmacokinetics and renin-inhibiting effect of aliskiren

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ABSTRACT***Purpose***

This study aimed to investigate possible effects of *ABCB1* haplotypes on the pharmacokinetics and renin-inhibiting effect of aliskiren.

Methods

Eleven healthy volunteers homozygous for the *ABCB1* c.1236C-c.2677G-c.3435C (CGC) haplotype and eleven homozygous for the c.1236T-c.2677T-c.3435T (TTT) haplotype ingested a single 150-mg dose of aliskiren. Plasma aliskiren concentrations were measured up to 72 h, its excretion into urine up to 12 h, and plasma renin activity up to 24 h.

Results

The *ABCB1* haplotypes had no significant effect on the pharmacokinetics or renin-inhibiting effect of aliskiren. The geometric mean ratio (95% confidence interval) of aliskiren peak plasma concentration and area under the plasma concentration-time curve from 0 h to infinity in participants homozygous for the TTT haplotype to those in participants homozygous for the CGC haplotype were 1.14 (0.66, 1.96; $P=0.631$) and 1.01 (0.58, 1.76; $P=0.960$), respectively.

Conclusions

These data suggest that the *ABCB1* c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes have no clinically meaningful effect on the pharmacokinetics of aliskiren.

KEYWORDS

Pharmacokinetics, Pharmacogenetics, *ABCB1* haplotypes, Aliskiren

INTRODUCTION

The direct renin inhibitor aliskiren is poorly absorbed and has a low oral bioavailability of 2-3% [1]. Aliskiren is mainly eliminated by biliary excretion [1]. In addition, about 0.4% of the oral dose is excreted unchanged into urine and about 1.4% undergoes oxidative biotransformation via CYP3A4 [1]. Aliskiren is a substrate of P-glycoprotein (also known as ABCB1) [2], an efflux transporter expressed in tissues with an excretory or barrier function [3, 4]. The P-glycoprotein and CYP3A4 inhibitors, itraconazole and cyclosporine [5-9], have raised the area under the plasma concentration-time curve (AUC) of aliskiren by about 6.5-fold and 5-fold, respectively [10, 11], supporting a major role of P-glycoprotein in limiting the intestinal absorption of aliskiren. Moreover, rifampicin, an inducer of P-glycoprotein and CYP3A4 [12, 13], has reduced the AUC of aliskiren by 56% [14].

A common synonymous single nucleotide polymorphism (SNP) in the *ABCB1* gene encoding P-glycoprotein, c.3435C>T, has been variably associated with reduced expression and function of P-glycoprotein *in vitro* and increased plasma concentrations of P-glycoprotein substrate drugs *in vivo* [15-17]. The mechanism underlying these associations is not completely understood, but it has been suggested that c.3435C>T affects *ABCB1* mRNA stability [18]. Alternatively, the associations may be explained by linkage disequilibrium between c.3435C>T and a functional sequence variation, or that c.3435C>T and sequence variations in linkage disequilibrium with it form a haplotype conferring altered P-glycoprotein activity [16, 19]. The *ABCB1* c.3435C>T

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4 SNP is in strong linkage disequilibrium with the c.1236C>T (synonymous) and
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6 c.2677G>T/A (p.Ala893Ser/Thr) SNPs within the same gene [17, 19, 20].
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11 The variant alleles c.1236T, c.2677T, and c.3435T are common in Europeans and
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13 European Americans, with reported frequencies of occurrence ranging from 39 to 46%,
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15 39 to 47%, and 54 to 60%, respectively [17, 19-22]. In the Finnish population, the two
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17 most common haplotypes based on these SNPs are c.1236T-c.2677T-c.3435T (TTT)
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19 and c.1236C-c.2677G-c.3435C (CGC), with frequencies of 42.7% and 34.4%,
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21 respectively [17]. The *ABCB1* TTT haplotype has been associated, for example, with
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23 increased plasma concentrations of and enhanced lipid-lowering response to simvastatin
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25 (acid) and atorvastatin, compared with the *ABCB1* CGC haplotype [17, 23, 24].
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33 Considerable interindividual variability exists in the pharmacokinetics of aliskiren [25],
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35 but the background of this variability is unknown. Our hypothesis was that genetic
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37 variability in *ABCB1* affects the pharmacokinetics of aliskiren. Therefore, we
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39 investigated the effects of the *ABCB1* CGC and TTT haplotypes on the
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41 pharmacokinetics and pharmacodynamics of aliskiren.
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METHODS

Subjects

Twenty-two healthy White Finnish volunteers participated in this study after giving written informed consent. They were recruited from a group of more than 700 subjects genotyped for the *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642) SNPs [17]. To minimize variability in aliskiren pharmacokinetics due to other genetic variants, all subjects were genotyped for the functionally significant *ABCB1* c.1199G>A (p.Ser400Asn; rs2229109) [17] and *CYP3A5**3 (g.6986A>G; rs776746) [26] alleles and only noncarriers of the *ABCB1* c.1199A and *CYP3A5* g.6986A (*CYP3A5* expressor) alleles were recruited.

Four women and seven men homozygous for the *ABCB1* CGC haplotype, mean \pm SD age 26 ± 4 years (range, 22-37 years), height 178 ± 9 cm (158-189 cm), and weight 74 ± 11 kg (63-95 kg), and five women and six men homozygous for the TTT haplotype, age 24 ± 2 years (21-27 years), height 175 ± 10 cm (158-186 cm), and weight 69 ± 12 kg (51-94 kg), participated in the study. Each participant's health was ascertained by medical history, clinical examination, and laboratory tests. None was on any continuous medication and none was a tobacco smoker.

Study design

The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District, and the National Agency for Medicines. Following an overnight fast, the subjects ingested a single 150-mg dose of aliskiren (Rasilez;

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4 Novartis, Horsham, UK) with 150 ml water. A standardized warm meal was served 4 h
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6 after aliskiren, and a standardized light meal after 7 h and 10 h. As a safety assessment,
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8 systolic and diastolic blood pressures were measured from the forearm with an
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10 automatic oscillometric blood pressure monitor (Omron M5-I, Omron Healthcare
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12 Europe BV, Hoofddorp, The Netherlands), with the participant in a sitting position,
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14 prior to and 2, 4, 7, 9, 12, and 24 h after aliskiren ingestion. The participants were under
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16 direct medical supervision for 12 h after the administration of aliskiren. No significant
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18 differences in blood pressures were observed between the haplotype groups (data not
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20 shown). Use of other drugs was prohibited for one week and use of grapefruit products
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22 for three days before aliskiren administration and 72 h thereafter.
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31 Timed blood samples (5 or 10 ml each) for aliskiren concentration measurements were
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33 drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) prior to and 0.5, 1,
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35 2, 3, 4, 5, 7, 9, 12, 24, 34, 48, and 72 h after aliskiren administration. Blood samples for
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37 the determination of plasma renin activity (5 ml each) were drawn before the
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39 administration of aliskiren and 4 h and 24 h thereafter into chilled EDTA tubes, which
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41 were placed on ice immediately after sampling. Plasma was separated within 30 min.
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44 Urine was collected up to 12 h after aliskiren ingestion. Urine aliquots and plasma were
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46 stored at -70°C until analysis.
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51 **Determination of aliskiren concentrations and renin activity**

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53 Plasma concentrations of aliskiren were quantified by use of solid phase extraction and
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55 liquid chromatography-tandem mass spectrometry (LC/MS/MS) [10]. An aliquot of 700
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57 μ l of each plasma sample was mixed with 100 μ l of 10% phosphoric acid and 50 μ l
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4 internal standard solution (1000 ng/ml acebutolol in 1:1 methanol:water). Then the
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6 mixture was loaded to the extraction cartridge (Oasis MCX 1cc/30 mg, Waters, Milford,
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8 MA, USA), which had been pre-conditioned with 1 ml of methanol and 1 ml of 1%
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10 acetic acid. The cartridge was subsequently washed with 1 ml of methanol and 1 ml of
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12 1% acetic acid. The analytes were eluted out with two times 0.6 ml of 2% ammonium
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14 hydroxide in methanol. The eluent was dried at 50°C under a nitrogen stream. The
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16 residue was dissolved in 100 µl of the mobile phase, and 15 µl of the sample was
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18 injected to the LC/MS/MS system. The chromatographic separation was carried out on
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20 an AtlantisT3 column (2.1×100 mm, 3 µm) protected by an AtlantisT3 (2.1×10 mm, 3
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22 µm) guard cartridge (Waters). The mobile phase consisted of (A) 2 mmol/l ammonium
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24 acetate (pH 4.90) and (B) methanol. The total running time was 15 minutes with the
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26 mobile phase gradient of 0.5 minutes at 70% A, 0.5 minutes to 5% A, 3 minutes at 5%
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28 A, 0.1 minutes to 70% A, and 10.9 minutes at 70% A. The flow rate of the mobile phase
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30 was 250 µl/min. The column temperature was 40°C. Mass spectra were obtained using
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32 an Applied Biosystems SCIEX API 2000 Q Trap mass spectrometer operated in
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34 positive ion mode (Sciex Division of MDS Inc, Toronto, ON, Canada). The ion
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36 transitions were mass-to-charge ratio (m/z) 552.4 to m/z 436.3 for aliskiren and m/z
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38 337.3 to m/z 116.3 for acebutolol.
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49 For the determination of aliskiren concentrations in urine, an aliquot of 300 µl of each
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51 urine sample was mixed with 300 µl of fresh frozen plasma, 100 µl of 10% phosphoric
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53 acid, and 50 µl internal standard solution (1000 ng/ml acebutolol in 1:1
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55 methanol:water). The extraction, chromatographic separation, and mass spectrometric
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57 detection were performed as described for plasma aliskiren. The lower limit of
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4 quantification was 0.25 ng/ml for plasma and 9 ng/ml for urine aliskiren. The
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6 calibration curve for plasma aliskiren was linear over the range 0.25-500 ng/ml
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8 ($r > 0.999$, weighting $1/x$) and for urine aliskiren over the range 9-1800 ng/ml ($r > 0.999$,
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10 weighting $1/x$). The extraction recoveries were $> 80\%$. The intra-day coefficients of
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12 variation were $\leq 1.2\%$ at relevant concentrations in plasma and $\leq 4.8\%$ in urine ($n=6$).
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14 Plasma renin activity was determined by radioimmunoassay of generated angiotensin I
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16 with a commercially available method (RENCTK; DiaSorin, Saluggia, Italy) at
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18 HUSLAB laboratory (Helsinki, Finland).
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26 **Pharmacokinetics and pharmacodynamics**

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28 Aliskiren pharmacokinetics were characterized by peak plasma concentration (C_{\max}),
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30 time to C_{\max} (t_{\max}), elimination half-life ($t_{1/2}$), AUC_{0-72} , $AUC_{0-\infty}$, the amount of aliskiren
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32 excreted into urine from 0 to 12 h (A_e), and the renal clearance (Cl_{renal}).
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35 Pharmacokinetic parameters were calculated with non-compartmental methods using
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37 MK-Model, version 5.0 (Biosoft, Cambridge, UK). The terminal log-linear part of each
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39 concentration-time curve was identified visually. The elimination rate constant (k_e) was
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41 determined by linear regression analysis of the log-linear part of the plasma drug
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43 concentration-time curve. The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln 2 / k_e$. The AUC
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45 values were calculated by a combination of the linear and log-linear trapezoidal rules
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47 with extrapolation to infinity, when appropriate, by division of the last measured
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49 concentration by k_e . The Cl_{renal} of aliskiren was calculated by the equation
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52 $Cl_{\text{renal}} = A_e / AUC_{0-12}$. Aliskiren pharmacodynamics were characterized by plasma renin
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54 activity at 4 h and 24 h after aliskiren intake.
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Statistical analysis

The data were analyzed using SPSS 17.0 (SPSS, Chicago, IL, USA). The C_{\max} , AUC, Ae, and Cl_{renal} data were logarithmically transformed before statistical analysis.

Statistical comparisons were made between the *ABCB1* haplotypes using analysis of variance (ANOVA). Body weight was set as a covariate for C_{\max} , AUC, and Ae [27].

The t_{\max} data were compared with the Mann-Whitney test. Differences were considered statistically significant when $P < 0.05$. The geometric mean ratio between the TTT and CGC homozygotes with 95% confidence interval (CI) was calculated for the C_{\max} , AUC, Ae, and Cl_{renal} values, and the mean difference with 95% CI for the $t_{1/2}$ and renin activity variables. The number of subjects was estimated to be sufficient to detect a 50% difference in the $AUC_{0-\infty}$ of aliskiren between the *ABCB1* CGC and TTT homozygotes, with a power of 80% (α -level 5%).

RESULTS

ABCB1 haplotypes had no significant effect on the pharmacokinetics or renin-inhibiting effect of aliskiren (Table 1, Fig. 1). The geometric mean ratio (95% CI) of aliskiren C_{\max} and $AUC_{0-\infty}$ in participants homozygous for the TTT haplotype to those in participants homozygous for the CGC haplotype were 1.14 (0.66, 1.96; $P=0.631$) and 1.01 (0.58, 1.76; $P=0.960$), respectively. As a *post hoc* analysis, fractional AUC values (AUC_{0-1} , AUC_{0-2} , AUC_{0-3} , AUC_{0-4} , and AUC_{0-5}) were also calculated for aliskiren, but no tendency for a difference between the haplotype groups was observed in these values ($P>0.3$, data not shown). Aliskiren C_{\max} , $AUC_{0-\infty}$, A_e , and Cl_{renal} varied 14.1-, 16.2-, 10.4-, and 3.1-fold, respectively, between individual participants.

DISCUSSION

In the present study, the *ABCB1* haplotypes had no significant effect on the pharmacokinetics or renin-inhibiting effect of the P-glycoprotein substrate aliskiren. However, these genetic results do not indicate that the P-glycoprotein transporter could not be otherwise important for the pharmacokinetics of aliskiren. *In vitro*, aliskiren is a high affinity substrate of P-glycoprotein (K_m 2.1 $\mu\text{mol/l}$), as evidenced by studies in P-glycoprotein-expressing Sf9 cell membrane vesicles [2]. *In vivo*, inhibitors and inducers of P-glycoprotein markedly affect aliskiren pharmacokinetics [10, 11, 14].

It is noteworthy that *ABCB1* genotypes have explained only a fraction of the variability in P-glycoprotein expression, and the effects of *ABCB1* genotypes and haplotypes on drug pharmacokinetics have been modest [15, 17, 28, 29]. For example, the AUCs of simvastatin acid and atorvastatin have been 60% and 55% larger, respectively, in subjects homozygous for the *ABCB1* TTT haplotype compared with subjects homozygous for the CGC haplotype [17]. Studies investigating the effects of the *ABCB1* c.3435C>T SNP on the pharmacokinetics of the P-glycoprotein prototype substrate digoxin have revealed conflicting results, with a meta-analysis suggesting that the *ABCB1* c.3435C>T SNP does not affect digoxin pharmacokinetics [12, 15, 28-31].

It has been suggested that the *ABCB1* c.3435C>T SNP, or its combination with the c.1236C>T or c.2677G>T/A SNP, may alter the substrate specificity of P-glycoprotein [16]. Therefore, the effect of the *ABCB1* genotype on the pharmacokinetics of different P-glycoprotein substrates may vary.

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4 The present study had sufficient power to detect a 50% difference in the $AUC_{0-\infty}$ of
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6 aliskiren between *ABCB1* haplotypes, and thus a small difference cannot be ruled out.
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8 However, it is unlikely that a smaller than 50% difference in aliskiren $AUC_{0-\infty}$ would be
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10 of clinical importance. For example, a 76% increase in aliskiren AUC by ketoconazole
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12 was not considered clinically relevant [2]. Nevertheless, the mean $AUC_{0-\infty}$ values of
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14 aliskiren were almost identical in the two haplotype groups.
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21 Similarly to previous reports [25], a high interindividual variability was observed in the
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23 C_{max} and AUC values of aliskiren. The present results suggest that common *ABCB1*
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25 haplotypes do not explain this variability. The large differences in aliskiren
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27 pharmacokinetics might be explained by variability in gastrointestinal physiology, and
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29 other genetic and non-genetic factors affecting the activity of P-glycoprotein or CYP3A,
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31 or other transporters or enzymes involved in aliskiren pharmacokinetics [2].
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37 In conclusion, the common *ABCB1* haplotypes c.1236C-c.2677G-c.3435C and c.1236T-
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39 c.2677T-c.3435T have no effects on the pharmacokinetics or pharmacodynamics of
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COMPETING INTEREST

None to declare.

For Peer Review

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Table 1 Pharmacokinetic variables of and plasma renin activity response to a single 150-mg oral dose of aliskiren in healthy subjects homozygous for the *ABCB1* c.1236C-c.2677G-c.3435C (CGC/CGC genotype; $n=11$) or c.1236T-c.2677T-c.3435T (TTT/TTT genotype; $n=11$) haplotype

Variable	<i>ABCB1</i> genotype		Geometric mean ratio (95% CI)	<i>P</i> value
	CGC/CGC	TTT/TTT		
Aliskiren				
C_{\max} (ng/ml)	98 (67, 143)	111 (76, 163)	1.14 (0.66, 1.96)	0.631
t_{\max} (h)	1 (0.5-5)	0.5 (0.5-5)		0.495
$t_{1/2}$ (h)	23.9 (19.0, 28.8)	22.5 (17.6, 27.4)	-1.4 (-8.3, 5.5)*	0.684
AUC ₀₋₇₂ (ng·h/ml)	419 (289, 608)	429 (296, 622)	1.02 (0.60, 1.74)	0.927
AUC _{0-∞} (ng·h/ml)	443 (301, 652)	449 (306, 661)	1.01 (0.58, 1.76)	0.960
Ae (mg)	0.499 (0.335, 0.742)	0.516 (0.347, 0.767)	1.03 (0.58, 1.83)	0.905
Cl _{renal} (l/h)	1.75 (1.48, 2.06)	1.71 (1.45, 2.01)	0.98 (0.77, 1.23)	0.842
Plasma renin activity (ng/ml/h)				
At baseline	1.73 (0.96, 2.50)	2.38 (1.61, 3.15)	0.66 (-0.43, 1.75)*	0.222
At 4 h	0.01 (-0.09, 0.11)	0.09 (-0.01, 0.19)	0.08 (-0.06, 0.22)*	0.251
At 24 h	1.08 (0.51, 1.65)	1.36 (0.79, 1.92)	0.28 (-0.52, 1.08)*	0.479

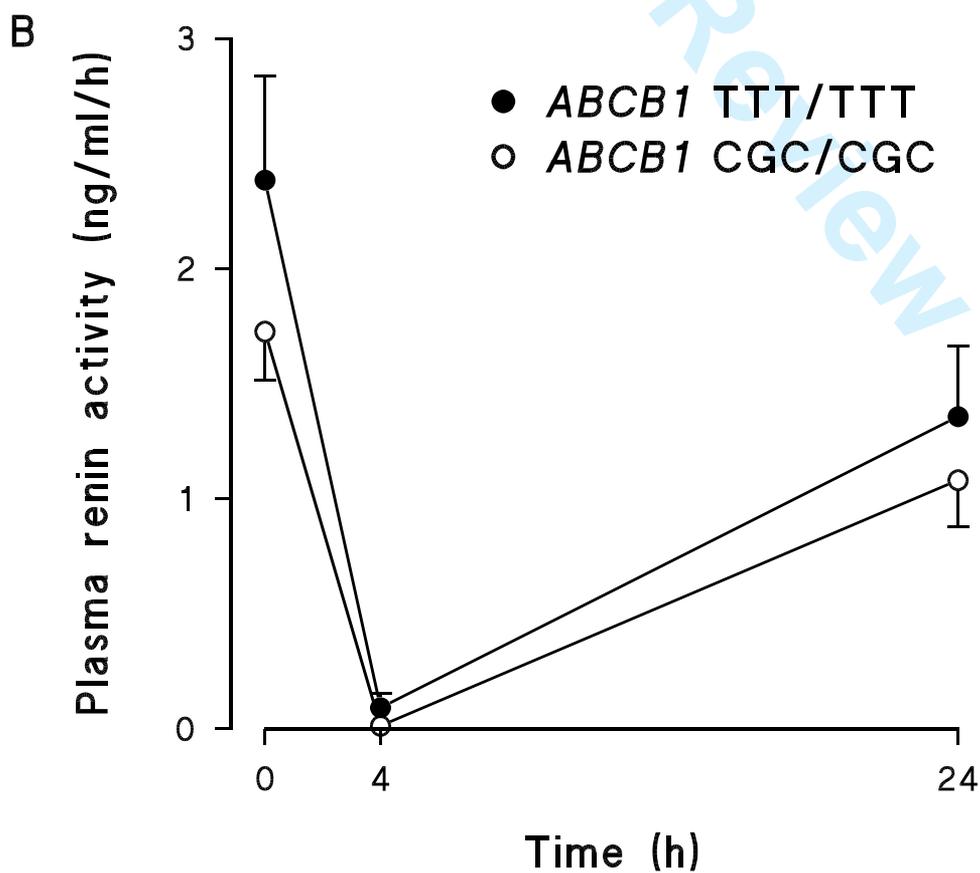
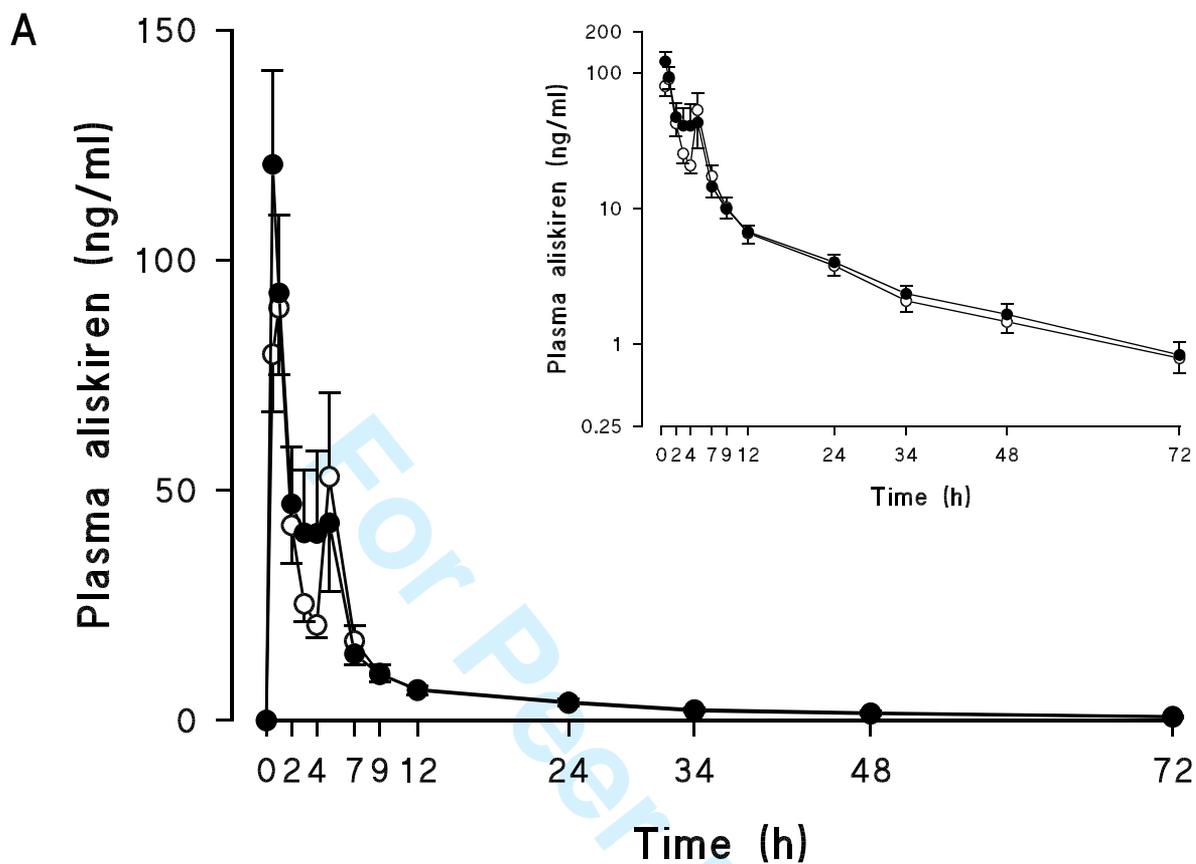
C_{\max} , AUC, and Ae data are estimated marginal mean (95% CI) adjusting for weight; $t_{1/2}$, Cl_{renal}, and plasma renin activity are estimated marginal mean (95% CI); t_{\max} are median (range). CI, Confidence interval; C_{\max} , peak plasma concentration; t_{\max} , time to C_{\max} ; $t_{1/2}$, elimination half-life; AUC₀₋₇₂, area under the plasma concentration-time curve from 0 to 72 h; AUC_{0-∞}, area under the plasma concentration-time curve from time 0 to infinity; Ae, amount excreted into urine within 12 h; Cl_{renal} renal clearance; Baseline, before the administration of aliskiren.

* Mean difference (95% CI).

FIGURE LEGEND

Fig. 1 Weight-adjusted mean \pm SEM plasma concentrations of aliskiren (A) and mean \pm SEM plasma renin activity (B) after a single 150-mg oral dose of aliskiren in subjects homozygous for the *ABCBI* c.1236C-c.2677G-c.3435C ($n=11$; *open circles*) or *ABCBI* c.1236T-c.2677T-c.3435T ($n=11$; *solid circles*) haplotype. The inset in part A shows aliskiren plasma concentration data on a semi-logarithmic scale

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Effect of *ABCB1* haplotypes on the pharmacokinetics and renin-inhibiting effect of aliskiren

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A revised manuscript ([EJCP 2010-0093](#)) for *European Journal of Clinical
Pharmacology*, including 21 pages, 1 table, and 1 figure ([April 2010](#)).

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ABSTRACT**Purpose**

This study aimed to investigate possible effects of *ABCB1* [haplotypes](#) on the pharmacokinetics and renin-inhibiting effect of aliskiren.

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Methods

Eleven healthy volunteers [homozygous for the *ABCB1* c.1236C-c.2677G-c.3435C \(CGC\) haplotype](#) and eleven [homozygous for the c.1236T-c.2677T-c.3435T \(TTT\) haplotype](#) ingested a single 150-mg dose of aliskiren. Plasma aliskiren concentrations were measured up to 72 h, its excretion into urine up to 12 h, and plasma renin activity up to 24 h.

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Results

The *ABCB1* [haplotypes](#) had no significant effect on the pharmacokinetics or renin-inhibiting effect of aliskiren. The geometric mean ratio (95% confidence interval) of aliskiren peak plasma concentration and area under the plasma concentration-time curve from 0 h to infinity in participants [homozygous for the TTT haplotype](#) to those in participants [homozygous for the CGC haplotype](#) were 1.14 (0.66, 1.96; $P=0.631$) and 1.01 (0.58, 1.76; $P=0.960$), respectively.

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Conclusions

These data suggest that the *ABCB1* c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes have no clinically meaningful effect on the pharmacokinetics of aliskiren.

KEYWORDS

Pharmacokinetics, Pharmacogenetics, *ABCB1* haplotypes, Aliskiren

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INTRODUCTION

The direct renin inhibitor aliskiren is poorly absorbed and has a low oral bioavailability of 2-3% [1]. Aliskiren is mainly eliminated by biliary excretion [1]. In addition, about 0.4% of the oral dose is excreted unchanged into urine and about 1.4% undergoes oxidative biotransformation via CYP3A4 [1]. Aliskiren is a substrate of P-glycoprotein (also known as ABCB1) [2], an efflux transporter expressed in tissues with an excretory or barrier function [3, 4]. The P-glycoprotein and CYP3A4 inhibitors, itraconazole and cyclosporine [5-9], have raised the area under the plasma concentration-time curve (AUC) of aliskiren by about 6.5-fold and 5-fold, respectively [10, 11], supporting a major role of P-glycoprotein in limiting the intestinal absorption of aliskiren. Moreover, rifampicin, an inducer of P-glycoprotein and CYP3A4 [12, 13], has reduced the AUC of aliskiren by 56% [14].

A common synonymous single nucleotide polymorphism (SNP) in the *ABCB1* gene encoding P-glycoprotein, c.3435C>T, has been variably associated with reduced expression and function of P-glycoprotein *in vitro* and increased plasma concentrations of P-glycoprotein substrate drugs *in vivo* [15-17]. The mechanism underlying these associations is not completely understood, but it has been suggested that c.3435C>T affects *ABCB1* mRNA stability [18]. Alternatively, the associations may be explained by linkage disequilibrium between c.3435C>T and a functional sequence variation, or that c.3435C>T and sequence variations in linkage disequilibrium with it form a haplotype conferring altered P-glycoprotein activity [16, 19]. The *ABCB1* c.3435C>T

Deleted: ABCB1 (also known as P-glycoprotein) is an efflux transporter expressed in tissues with an excretory or barrier function [1, 2].

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SNP is in strong linkage disequilibrium with the c.1236C>T (synonymous) and c.2677G>T/A (p.Ala893Ser/Thr) SNPs within the same gene [17, 19, 20].

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The variant alleles c.1236T, c.2677T, and c.3435T are common in Europeans and European Americans, with reported frequencies of occurrence ranging from 39 to 46%, 39 to 47%, and 54 to 60%, respectively [17, 19-22]. In the Finnish population, the two

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most common haplotypes based on these SNPs are c.1236T-c.2677T-c.3435T (TTT)

and c.1236C-c.2677G-c.3435C (CGC), with frequencies of 42.7% and 34.4%,

respectively [17]. The *ABCB1* TTT haplotype has been associated, for example, with

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increased plasma concentrations of and enhanced lipid-lowering response to simvastatin

(acid) and atorvastatin, compared with the *ABCB1* CGC haplotype [17, 23, 24].

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Considerable interindividual variability exists in the pharmacokinetics of aliskiren [25],

but the background of this variability is unknown. Our hypothesis was that genetic

variability in *ABCB1* affects the pharmacokinetics of aliskiren. Therefore, we

investigated the effects of the *ABCB1* CGC and TTT haplotypes on the

pharmacokinetics and pharmacodynamics of aliskiren.

Deleted: The antihypertensive drug aliskiren is a substrate of ABCB1 [13]. It is poorly absorbed and has a low oral bioavailability of 2-3% [14]. Aliskiren is mainly eliminated by biliary excretion [14]. In addition, about 0.4% of the oral dose is excreted unchanged into urine and about 1.4% undergoes oxidative biotransformation via CYP3A4 [14]. The ABCB1 and CYP3A4 inhibitors, itraconazole and cyclosporine [15-19], have raised the AUC of aliskiren by about 6.5-fold and 5-fold, respectively [20, 21], supporting a major role of ABCB1 in limiting the intestinal absorption of aliskiren. Moreover, rifampicin, an inducer of ABCB1 and CYP3A4 [22, 23], has reduced the AUC of aliskiren by 56% [24].¶

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METHODS

Subjects

Twenty-two healthy White [Finnish](#) volunteers participated in this study after giving written informed consent. They were recruited from a group of more than 700 subjects genotyped for the *ABCB1* c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642) SNPs [\[17\]](#). To minimize variability in aliskiren pharmacokinetics due to other genetic variants, all subjects were genotyped for the functionally significant *ABCB1* c.1199G>A (p.Ser400Asn; rs2229109) [\[17\]](#) and *CYP3A5**3 (g.6986A>G; rs776746) [\[26\]](#) alleles and only noncarriers of the *ABCB1* c.1199A and *CYP3A5* g.6986A (*CYP3A5* expressor) alleles were recruited.

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Four women and seven men [homozygous for the *ABCB1* CGC haplotype](#), mean \pm SD age 26 ± 4 years ([range, 22-37 years](#)), height 178 ± 9 cm ([158-189 cm](#)), and weight 74 ± 11 kg ([63-95 kg](#)), and five women and six men [homozygous for the TTT haplotype](#), age 24 ± 2 years ([21-27 years](#)), height 175 ± 10 cm ([158-186 cm](#)), and weight 69 ± 12 kg ([51-94 kg](#)), participated in the study. Each participant's health was ascertained by medical history, clinical examination, and laboratory tests. None was on any continuous medication and none was a tobacco smoker.

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Study design

The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District, and the National Agency for Medicines. Following an overnight fast, the subjects ingested a single 150-mg dose of aliskiren (Rasilez;

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3 Novartis, Horsham, UK) with 150 ml water. A standardized warm meal was served 4 h
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5 after aliskiren, and a standardized light meal after 7 h and 10 h. As a safety assessment,
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7 systolic and diastolic blood pressures were measured from the forearm with an
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9 automatic oscillometric blood pressure monitor (Omron M5-I, Omron Healthcare
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11 Europe BV, Hoofddorp, The Netherlands), with the participant in a sitting position,
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13 prior to and 2, 4, 7, 9, 12, and 24 h after aliskiren ingestion. The participants were under
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15 direct medical supervision for 12 h after the administration of aliskiren. No significant
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17 differences in blood pressures were observed between the haplotype groups (data not
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19 shown). Use of other drugs was prohibited for one week and use of grapefruit products
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21 for three days before aliskiren administration and 72 h thereafter.

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25 Timed blood samples (5 or 10 ml each) for aliskiren concentration measurements were
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27 drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) prior to and 0.5, 1,
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29 2, 3, 4, 5, 7, 9, 12, 24, 34, 48, and 72 h after aliskiren administration. Blood samples for
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31 the determination of plasma renin activity (5 ml each) were drawn before the
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33 administration of aliskiren and 4 h and 24 h thereafter into chilled EDTA tubes, which
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35 were placed on ice immediately after sampling. Plasma was separated within 30 min.
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37 Urine was collected up to 12 h after aliskiren ingestion. Urine aliquots and plasma were
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39 stored at -70°C until analysis.

Deleted: Timed ethylenediaminetetraacetic acid blood samples (5-15 ml each) were drawn prior to and up to 72 h after aliskiren ingestion. Plasma was separated within 30 min. Urine was collected up to 12 h after aliskiren ingestion. The samples were stored at -70°C until analysis. ¶

40 41 42 43 **Determination of aliskiren concentrations and renin activity**

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45 Plasma concentrations of aliskiren were quantified by use of solid phase extraction and
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47 liquid chromatography-tandem mass spectrometry (LC/MS/MS) [10]. An aliquot of 700
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49 µl of each plasma sample was mixed with 100 µl of 10% phosphoric acid and 50 µl
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3 internal standard solution (1000 ng/ml acebutolol in 1:1 methanol:water). Then the
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5 mixture was loaded to the extraction cartridge (Oasis MCX 1cc/30 mg, Waters, Milford,
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7 MA, USA), which had been pre-conditioned with 1 ml of methanol and 1 ml of 1%
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9 acetic acid. The cartridge was subsequently washed with 1 ml of methanol and 1 ml of
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11 1% acetic acid. The analytes were eluted out with two times 0.6 ml of 2% ammonium
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13 hydroxide in methanol. The eluent was dried at 50°C under a nitrogen stream. The
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15 residue was dissolved in 100 µl of the mobile phase, and 15 µl of the sample was
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17 injected to the LC/MS/MS system. The chromatographic separation was carried out on
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19 an AtlantisT3 column (2.1×100 mm, 3 µm) protected by an AtlantisT3 (2.1×10 mm, 3
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21 µm) guard cartridge (Waters). The mobile phase consisted of (A) 2 mmol/l ammonium
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23 acetate (pH 4.90) and (B) methanol. The total running time was 15 minutes with the
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25 mobile phase gradient of 0.5 minutes at 70% A, 0.5 minutes to 5% A, 3 minutes at 5%
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27 A, 0.1 minutes to 70% A, and 10.9 minutes at 70% A. The flow rate of the mobile phase
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29 was 250 µl/min. The column temperature was 40°C. Mass spectra were obtained using
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31 an Applied Biosystems SCIEX API 2000 Q Trap mass spectrometer operated in
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33 positive ion mode (Sciex Division of MDS Inc, Toronto, ON, Canada). The ion
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35 transitions were mass-to-charge ratio (m/z) 552.4 to m/z 436.3 for aliskiren and m/z
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37 337.3 to m/z 116.3 for acebutolol.
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41 For the determination of aliskiren concentrations in urine, an aliquot of 300 µl of each
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43 urine sample was mixed with 300 µl of fresh frozen plasma, 100 µl of 10% phosphoric
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45 acid, and 50 µl internal standard solution (1000 ng/ml acebutolol in 1:1
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47 methanol:water). The extraction, chromatographic separation, and mass spectrometric
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49 detection were performed as described for plasma aliskiren. The lower limit of
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Deleted: Aliskiren plasma and urine concentrations were quantified using an Applied Biosystems SCIEX Q Trap LC/MS/MS system (Sciex Division of MDS, Toronto, ON, Canada) [20]. Acebutolol served as an internal standard.

quantification was 0.25 ng/ml for plasma and 9 ng/ml for urine aliskiren. The calibration curve for plasma aliskiren was linear over the range 0.25-500 ng/ml ($r > 0.999$, weighting $1/x$) and for urine aliskiren over the range 9-1800 ng/ml ($r > 0.999$, weighting $1/x$). The extraction recoveries were $> 80\%$. The intra-day coefficients of variation were $\leq 1.2\%$ at relevant concentrations in plasma and $\leq 4.8\%$ in urine ($n=6$).

Plasma renin activity was determined by radioimmunoassay of generated angiotensin I with a commercially available method (RENCTK; DiaSorin, Saluggia, Italy) at HUSLAB laboratory (Helsinki, Finland).

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Pharmacokinetics and pharmacodynamics

Aliskiren pharmacokinetics were characterized by peak plasma concentration (C_{max}), time to C_{max} (t_{max}), elimination half-life ($t_{1/2}$), AUC_{0-72} , $AUC_{0-\infty}$, the amount of aliskiren excreted into urine from 0 to 12 h (A_e), and the renal clearance (Cl_{renal}).

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Pharmacokinetic parameters were calculated with non-compartmental methods using MK-Model, version 5.0 (Biosoft, Cambridge, UK). The terminal log-linear part of each concentration-time curve was identified visually. The elimination rate constant (k_e) was determined by linear regression analysis of the log-linear part of the plasma drug concentration-time curve. The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln 2/k_e$. The AUC values were calculated by a combination of the linear and log-linear trapezoidal rules with extrapolation to infinity, when appropriate, by division of the last measured concentration by k_e . The Cl_{renal} of aliskiren was calculated by the equation $Cl_{renal} = A_e/AUC_{0-12}$. Aliskiren pharmacodynamics were characterized by plasma renin activity at 4 h and 24 h after aliskiren intake.

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Statistical analysis

The data were analyzed using SPSS 17.0 (SPSS, Chicago, IL, USA). The C_{\max} , AUC, Ae, and Cl_{renal} data were logarithmically transformed before statistical analysis.

Statistical comparisons were made between the *ABCB1* [haplotypes](#) using analysis of variance (ANOVA). Body weight was set as a covariate for C_{\max} , AUC, and Ae [27].

The t_{\max} data were compared with the Mann-Whitney test. Differences were considered

statistically significant when $P < 0.05$. The geometric mean ratio between the TTT and

CGC [homozygotes](#) with 95% confidence interval (CI) was calculated for the C_{\max} ,

AUC, Ae, and Cl_{renal} values, and the mean difference with 95% CI for the $t_{1/2}$ and renin

activity variables. The number of subjects was estimated to be sufficient to detect a 50%

difference in the $AUC_{0-\infty}$ of aliskiren between the *ABCB1* CGC and TTT [homozygotes](#),

with a power of 80% (α -level 5%).

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RESULTS

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ABCB1 [haplotypes](#) had no significant effect on the pharmacokinetics or renin-inhibiting effect of aliskiren (Table 1, Fig. 1). The geometric mean ratio (95% CI) of aliskiren C_{\max} and $AUC_{0-\infty}$ in participants [homozygous for the TTT haplotype](#) to those in participants [homozygous for the CGC haplotype](#) were 1.14 (0.66, 1.96; $P=0.631$) and 1.01 (0.58, 1.76; $P=0.960$), respectively. As a *post hoc* analysis, fractional AUC values (AUC_{0-1} , AUC_{0-2} , AUC_{0-3} , AUC_{0-4} , and AUC_{0-5}) were also calculated for aliskiren, but no tendency for a difference between the [haplotype](#) groups was observed in these values ($P>0.3$, data not shown). Aliskiren C_{\max} , $AUC_{0-\infty}$, A_e , and Cl_{renal} varied 14.1-, 16.2-, 10.4-, and 3.1-fold, respectively, between individual participants.

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DISCUSSION

In the present study, the *ABCB1* [haplotypes](#) had no significant effect on the pharmacokinetics or renin-inhibiting effect of the [P-glycoprotein](#) substrate aliskiren.

However, these genetic results do not indicate that the [P-glycoprotein](#) transporter could not be otherwise important for the pharmacokinetics of aliskiren. *In vitro*, aliskiren is a

high affinity substrate of [P-glycoprotein](#) (K_m 2.1 $\mu\text{mol/l}$), as evidenced by studies in [P-glycoprotein](#)-expressing Sf9 cell membrane vesicles [2]. *In vivo*, inhibitors and inducers of [P-glycoprotein](#) markedly affect aliskiren pharmacokinetics [10, 11, 14].

It is noteworthy that *ABCB1* genotypes have explained only a fraction of the variability in [P-glycoprotein](#) expression, and the effects of *ABCB1* genotypes and haplotypes on drug pharmacokinetics have been modest [15, 17, 28, 29]. For example, the AUCs of simvastatin acid and atorvastatin have been 60% and 55% larger, respectively, in

subjects [homozygous for](#) the *ABCB1* TTT [haplotype](#) compared with subjects [homozygous for](#) the CGC [haplotype](#) [17]. Studies investigating the effects of the *ABCB1* c.3435C>T SNP on the pharmacokinetics of the [P-glycoprotein](#) prototype substrate digoxin have revealed conflicting results, with a meta-analysis suggesting that the *ABCB1* c.3435C>T SNP does not affect digoxin pharmacokinetics [12, 15, 28-31].

It has been suggested that the *ABCB1* c.3435C>T SNP, or its combination with the c.1236C>T or c.2677G>T/A SNP, may alter the substrate specificity of [P-glycoprotein](#) [16]. Therefore, the effect of the *ABCB1* genotype on the pharmacokinetics of different [P-glycoprotein](#) substrates may vary.

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3 The present study had sufficient power to detect a 50% difference in the $AUC_{0-\infty}$ of
4 aliskiren between *ABCB1* [haplotypes](#), and thus a small difference cannot be ruled out.

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7 However, it is unlikely that a smaller than 50% difference in aliskiren $AUC_{0-\infty}$ would be
8 of clinical importance. For example, a 76% increase in aliskiren AUC by ketoconazole
9 was not considered clinically relevant [2]. Nevertheless, the mean $AUC_{0-\infty}$ values of
10 aliskiren were almost identical in the two [haplotype](#) groups.

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17 Similarly to previous reports [25], a high interindividual variability was observed in the
18 C_{max} and AUC values of aliskiren. The present results suggest that common *ABCB1*
19 haplotypes do not explain this variability. The large differences in aliskiren
20 pharmacokinetics might be explained by variability in gastrointestinal physiology, and
21 other genetic and non-genetic factors affecting the activity of [P-glycoprotein](#) or CYP3A,
22 or other transporters or enzymes involved in aliskiren pharmacokinetics [2].

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31 In conclusion, the common *ABCB1* haplotypes c.1236C-c.2677G-c.3435C and c.1236T-
32 c.2677T-c.3435T have no effects on the pharmacokinetics or pharmacodynamics of
33 aliskiren.
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COMPETING INTEREST

None to declare.

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Table 1 Pharmacokinetic variables of and plasma renin activity response to a single 150-mg oral dose of aliskiren in healthy subjects homozygous for the *ABCB1* c.1236C-c.2677G-c.3435C (CGC/CGC genotype; $n=11$) or c.1236T-c.2677T-c.3435T (TTT/TTT genotype; $n=11$) haplotype

Variable	<i>ABCB1</i> genotype		Geometric mean ratio (95% CI)	<i>P</i> value
	CGC/CGC	TTT/TTT		
Aliskiren				
C_{max} (ng/ml)	98 (67, 143)	111 (76, 163)	1.14 (0.66, 1.96)	0.631
t_{max} (h)	1 (0.5-5)	0.5 (0.5-5)		0.495
$t_{1/2}$ (h)	23.9 (19.0, 28.8)	22.5 (17.6, 27.4)	-1.4 (-8.3, 5.5)*	0.684
AUC ₀₋₇₂ (ng·h/ml)	419 (289, 608)	429 (296, 622)	1.02 (0.60, 1.74)	0.927
AUC _{0-∞} (ng·h/ml)	443 (301, 652)	449 (306, 661)	1.01 (0.58, 1.76)	0.960
Ae (mg)	0.499 (0.335, 0.742)	0.516 (0.347, 0.767)	1.03 (0.58, 1.83)	0.905
Cl _{renal} (l/h)	1.75 (1.48, 2.06)	1.71 (1.45, 2.01)	0.98 (0.77, 1.23)	0.842
Plasma renin activity (ng/ml/h)				
At baseline	1.73 (0.96, 2.50)	2.38 (1.61, 3.15)	0.66 (-0.43, 1.75)*	0.222
At 4 h	0.01 (-0.09, 0.11)	0.09 (-0.01, 0.19)	0.08 (-0.06, 0.22)*	0.251
At 24 h	1.08 (0.51, 1.65)	1.36 (0.79, 1.92)	0.28 (-0.52, 1.08)*	0.479

C_{max} , AUC, and Ae data are estimated marginal mean (95% CI) adjusting for weight; $t_{1/2}$, Cl_{renal}, and plasma renin activity are estimated marginal mean (95% CI); t_{max} are median (range). CI, Confidence interval; C_{max} , peak plasma concentration; t_{max} , time to C_{max} ; $t_{1/2}$, elimination half-life; AUC₀₋₇₂, area under the plasma concentration-time curve from 0 to 72 h; AUC_{0-∞}, area under the plasma concentration-time curve from time 0 to infinity; Ae, amount excreted into urine within 12 h; Cl_{renal} renal clearance; Baseline, before the administration of aliskiren.

* Mean difference (95% CI).

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FIGURE LEGEND

Fig. 1 Weight-adjusted mean ± SEM plasma concentrations of aliskiren (A) and mean ± SEM plasma renin activity (B) after a single 150-mg oral dose of aliskiren in subjects

homozygous for the ABCB1 c.1236C-c.2677G-c.3435C (n=11; open circles) or ABCB1 c.1236T-c.2677T-c.3435T (n=11; solid circles) haplotype. The inset in part A shows aliskiren plasma concentration data on a semi-logarithmic scale

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