Universidad de Salamanca
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Optimización de la terapia con lopinavir/ritonavir en pacientes infectados por el VIH: aplicación de criterios farmacocinéticos y farmacogenéticos.

Elena López Aspiroz
Tesis Doctoral
2011

UNIVERSIDAD DE SALAMANCA

FACULTAD DE FARMACIA
DEPARTAMENTO DE FARMACIA Y TECNOLOGÍA FARMACÉUTICA

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La doctora Dña. MARÍA JOSÉ GARCÍA SÁNCHEZ, Catedrática del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Salamanca, la doctora Dña. DOLORES SANTOS BUELGA, Profesora Titular del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Salamanca y el doctor D. SALVADOR CABRERA FIGUEROA, Profesor Auxiliar del Instituto de Farmacia de la Universidad Austral de Chile

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En calidad de directores de la Tesis cuyo título es **"Optimización de la Terapia con Lopinavir/Ritonavir en Pacientes Infectados por el VIH: Aplicación de Criterios Farmacocinéticos y Farmacogenéticos"** realizada por la Licenciada en Farmacia Dña. Elena López Aspiroz , consideran concluido el trabajo y autorizan su presentación a fin de que pueda ser juzgado por el Tribunal correspondiente.

Y para que así conste, firman el presente certificado en Salamanca, a diecinueve de diciembre de dos mil once.

Fdo: Mª José García Sánchez

Fdo: Dolores Santos Buelga

Fdo: Salvador Cabrera Figueroa

En la vida no se puede tener todo, sin embargo, es necesario aspirar a ello,

porque la felicidad no es una meta sino un estilo de vida

Federico Moccia

AGRADECIMIENTOS

A mis directores de tesis, el Dr. Salvador Cabrera Figueroa, la Dra. Mª José García Sánchez y la Dra. Dolores Santos Buelga, por ofrecerme la oportunidad de llevar a cabo este proyecto, su entrega y dedicación, sus buenos consejos, su cariñoso trato y la confianza depositada en mí.

Al Dr. Alfonso Domínguez-Gil Hurlé, por apoyar incondicionalmente esta investigación, poner a mi alcance todos los recursos que han sido necesarios en cada momento y transmitir su constante entusiasmo e interés.

A los compañeros del Centro de Medicina Genómica de Santiago de Compostela, ya que ha sido una experiencia excelente el poder trabajar conjuntamente con ellos para profundizar en el conocimiento de la genética y la estadística, por su esfuerzo, profesionalidad y la calidad humana demostrada durante todo este tiempo.

A las farmacéuticas de la Unidad de Pacientes Externos del Hospital Universitario de Salamanca, Mª Paz Valverde, Almudena Sánchez y Begoña Domínguez, por ayudarme siempre en todo lo que ha sido posible, su constante apoyo y recibirme siempre con una sonrisa.

A todos mis compañeros del Servicio de Farmacia del Hospital Universitario de Salamanca, en especial a l os residentes y a Miguel González, por las distintas colaboraciones en este trabajo, su amistad y compañerismo, por los buenos momentos vividos y los ánimos que me han transmitido a lo largo de todo este tiempo.

A los compañeros del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Salamanca, por el trabajo experimental realizado, el cual ha sido fundamental para poder desarrollar este proyecto, así como por las gestiones administrativas necesarias.

Al equipo de Enfermedades Infecciosas del Hospital Universitario de Salamanca, especialmente a Tachy, por su colaboración en este trabajo, su profesionalidad y la buena disposición mostrada en todo momento.

A mis padres, Laura, Alfonso, Francis y mis amigos, por sus ánimos y la ayuda técnica durante la realización de este trabajo.

ÍNDICE

I. OBJETIVOS

✓ Revisar la información existente sobre los aspectos farmacocinéticos y farmacogenéticos aplicables a la utilización clínica de lopinavir/ritonavir (LPV/r) como componente del tratamiento antirretroviral de pacientes infectados por el VIH, así como las principales estrategias utilizadas en la optimización del tratamiento con este fármaco.

✓ Identificar y caracterizar, en la población de pacientes VIH+ analizada, los polimorfismos en genes codificantes de las principales proteínas responsables del metabolismo, transporte, eficacia y toxicidad de LPV/r, con el fin de establecer la potencial influencia de dichos polimorfismos sobre el comportamiento del fármaco y su aplicabilidad en la práctica clínica.

✓ Desarrollar y validar en pacientes VIH+ un modelo farmacocinético/ farmacogenético (PK/PG) poblacional que refleje el comportamiento cinético de LPV/r incluyendo los factores demográficos, antropométricos, clínicos y genéticos que influyan significativamente en su farmacocinética.

✓ Implementar el modelo PK/PG poblacional que se pretende desarrollar en un programa de farmacocinética clínica para estimar de forma individual, utilizando algoritmos Bayesianos, los parámetros farmacocinéticos de los pacientes VIH+ monitorizados en un centro hospitalario con el fin de optimizar la posología con LPV/r.

II. INTRODUCCIÓN

NUEVAS ESTRATEGIAS EN LA OPTIMIZACIÓN POSOLÓGICA DE LOPINAVIR/RITONAVIR EN PACIENTES INFECTADOS POR EL VIH

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RESUMEN

Lopinavir/ritonavir (LPV/r) ha demostrado eficacia virológica e inmunológica en el tratamiento antirretroviral (TAR) combinado, tanto en pacientes naïve como pretratados. Además presenta una alta barrera genética al desarrollo de resistencias y su perfil de tolerancia es aceptable, aunque son frecuentes alteraciones gastrointestinales y del perfil lipídico.

Se revisan diferentes estrategias utilizadas en la optimización del TAR con este fármaco en la práctica clínica diaria, haciendo especial incidencia en la monitorización de concentraciones plasmáticas de LPV/r y la caracterización farmacogenética de las principales isoenzimas responsables de su metabolismo y transporte. En este sentido la correlación del genotipo con el fenotipo establecida en la monitorización de niveles de LPV/r facilitaría la individualización posológica de los tratamientos con este fármaco. Así mismo, se revisa la estrategia de simplificación del tratamiento a monoterapia lo que permitiría incrementar la seguridad y disminuir los costes.

PALABRAS CLAVES

Lopinavir/ritonavir, VIH/SIDA, monitorización de niveles de fármacos, farmacogenética, monoterapia.

ABSTRACT

Lopinavir/ritonavir (LPV/r) has demonstrated virological and immunological efficacy in the combined antiretroviral treatment (cART), in both naïve and experienced patients. Furthermore, LPV/r showed a high barrier to the development of resistance. Although generally well tolerated, adverse gastrointestinal side effects and metabolic disorders are frequent.

Different tools that are used to optimize the cART with this drug in the daily clinical practice, emphasizing the therapeutic drug monitoring (TDM) of LPV/r and the genetic analysis of the main enzymes responsible for the metabolism and transport, are reviewed. The relationship between phenotype and genotype, established through TDM, could be useful for the physician to improve the clinical management of the HIV infection, due to the possibility of individualize the dose with this drug. It is also reviewed the monotherapy as a new strategy used in the simplification of the therapy with this drug, which could increase security and reduce costs.

KEYWORDS

Lopinavir/ritonavir, HIV/AIDS, therapeutic drug monitoring, pharmacogenetics, monotherapy.

INTRODUCCIÓN

El síndrome de inmunodeficiencia adquirida continúa siendo una prioridad sanitaria mundial, ya que es una de las primeras causas de muerte en los países en vías de desarrollo[1]. Aunque los efectos beneficiosos del tratamiento antirretroviral (TAR) combinado son indiscutibles, con frecuencia los acontecimientos adversos asociados a la terapia farmacológica pueden comprometer la calidad de vida y afectar la adherencia.

La introducción de los inhibidores de la proteasa (IPs) a mediados de la década de los 90 como tercer componente del TAR mejoró radicalmente los resultados del tratamiento de la infección por el VIH. La aparición de lopinavir (LPV) como el primer IP coformulado con el potenciador ritonavir (RTV) en el año 2001, constituyó uno de los grandes avances en la historia del TAR[2,3], lo que ha contribuido de manera importante a que LPV potenciado con RTV (LPV/r) desplazara al resto de IPs de primera generación[4].

En el momento actual, aunque se continúa trabajando en el diseño y desarrollo de nuevos fármacos dirigidos a otras dianas del VIH, también existen investigaciones paralelas enfocadas hacia la optimización de la terapia con el arsenal terapéutico actualmente disponible. La farmacocinética (PK) y la farmacogenética (PG) son disciplinas que paulatinamente están demostrando su utilidad en la optimización del TAR, especialmente en los centros hospitalarios que cuentan con la tecnología necesaria para su aplicación en la práctica clínica habitual. De hecho, existen documentos de consenso y publicaciones científicas que avalan la utilidad de la monitorización de niveles de fármacos (*Therapeutic Drug Monitoring*: TDM) antirretrovirales (ARTs) en ciertos escenarios terapéuticos[5-11] y de algunas determinaciones genéticas, como por ejemplo el HLA B*5701, CYP2B6*6 y SLCO1B1*4, para el tratamiento con abacavir[5-9], efavirenz[10-14] y LPV/r[15], respectivamente. En este documento se pretende revisar la

información disponible sobre la utilización de estas herramientas PK y PG aplicadas a LPV/r, así como las nuevas estrategias en los esquemas de tratamiento enfocados hacia la utilización de LPV/r en monoterapia[16] y a la administración de una única dosis diaria[17].

PROPIEDADES FARMACODINÁMICAS

La proteasa del VIH es una proteína compuesta por 99 aminoácidos y es responsable de la maduración de las partículas del virus. En el VIH se originan tres grandes precursores codificados por los genes: *env*, *gag* y *pol*. Las poliproteínas resultantes *gag* y *pol*, son procesadas por la proteasa del VIH, que cataliza la escisión de estos precursores polipeptídicos en subunidades funcionales para la formación de la cápside viral y enzimas virales, necesarias para dar un virión infeccioso[18]. Los IPs actúan como inhibidores competitivos, que se unen directamente a la proteasa del VIH-1 y VIH-2, bloqueando así la escisión de las poliproteínas *gag* y *pol*, previniendo la ruptura posterior de los polipéptidos y por tanto la maduración del virus, dando lugar a viriones no infecciosos[18]. Aunque todos los IPs tienen este mecanismo de acción, presentan entre ellos importantes diferencias en la eficacia y en el perfil de eventos adversos[5,7].

PROPIEDADES FARMACOCINÉTICAS

El comportamiento PK de LPV/r condiciona las concentraciones que alcanza este fármaco en los diferentes órganos y tejidos así como su permanencia en el organismo. Considerando que las concentraciones de fármaco constituyen una variable subrogada de la respuesta, resulta de gran interés conocer sus características PK.

La absorción de LPV/r tras su administración en forma de cápsulas blandas o solución, formulaciones inicialmente comercializadas (Kaletra®, Laboratorios Abbott), estaba

claramente influenciada por la presencia de alimentos. Así, tras la administración de una dosis única de 400/100 mg en cápsulas blandas con una dieta de moderado contenido graso, la concentración máxima (C_{max}) y el área bajo la curva (ABC) de LPV se incrementaron un 23% y 48%, respectivamente, y un 54% y 80% para la solución oral[2], con respecto a la administración en ayuno. Por ello, se aconseja su administración con las comidas con el fin de mejorar su biodisponibilidad, la cual presenta una elevada variabilidad intra e interindividual. Con objeto de evitar estos problemas, en 2006 se aprobó una nueva formulación en comprimidos con la tecnología de *melt-extrusion*, que ha mostrado una exposición a ambos fármacos similar, independientemente de la presencia o no de alimentos, y una absorción más rápida[2,19]. Con esta formulación a la dosis de 400/100 mg dos veces al día en régimen de dosis múltiple se alcanza, aproximadamente a las 4 horas de la administración, una concentración máxima media en el estado de equilibrio (C^{ss}_{max}) de 12,3 ± 5,4 mcg/mL[2].

LPV se une a proteínas plasmáticas, principalmente a la α_1-glicoproteína ácida y a la albúmina, en aproximadamente un 98-99%[2], presentando a pesar de ello un elevado volumen aparente de distribución, con valores entre 70-130 L[15,20,21] que pone de manifiesto su elevado grado de acceso a órganos y tejidos. Así, esta fracción de LPV no unido a proteínas plasmáticas (1-2%) es capaz de atravesar la barrera hematoencefálica, siendo la concentración mínima media en el estado de equilibrio (C^{ss}_{min}) de LPV en fluido cerebroespinal superior al cociente inhibitorio 50% para el VIH, siendo la relación concentración líquido cefalorraquídeo/plasma de 0,23[22]. Sin embargo, su acceso al aparato reproductor masculino y femenino, así como a la leche materna, es limitado[23-26] y apenas atraviesa la barrera placentaria, por lo que la exposición al fármaco del feto es mínima[27].

El mecanismo fundamental de eliminación de LPV es la biotransformación a nivel hepático y en menor grado, intestinal, lo que podría condicionar su baja biodisponibilidad por un marcado efecto de primer paso. Así, LPV experimenta una rápida e intensa oxidación vía citocromo P450 (CYP), principalmente por el CYP3A (CYP3A4 y CYP3A5), no existiendo evidencias de que presente reacciones de conjugación en fase II[2,28]. Se han identificado unos 13 metabolitos que se eliminan a través de la orina y las heces[2,28].

LPV se asocia con RTV, conocido inhibidor enzimático de las isoenzimas CYP3A4 y CYP3A5, con el objetivo de reducir su metabolismo y mantener concentraciones terapéuticas con menores dosis, efecto conocido como "potenciación"[29,30]. No obstante, RTV también es capaz de inducir su propio metabolismo así como otras enzimas metabólicas minoritarias responsables de la biotransformación de LPV[29,30]. Aproximadamente un 20% y menos del 3% de la dosis de LPV en forma inalterada se excreta por vía fecal y renal, respectivamente[2].

Cuando LPV/r se administra en régimen de dosis múltiples (400/100 mg) dos veces al día (BID) se alcanzan C^{ss}_{min} de LPV de 8,1 ± 5,7 mcg/mL[2]. Su grado medio de exposición evaluado mediante el ABC en un intervalo de 12 horas resultó ser de 113,2 ± 60,5 mcg•h/mL[2]. En esta situación de equilibrio, se han estimado valores medios para la semivida de eliminación y el aclaramiento oral de LPV, de 5-6 horas y 6 -7 L/h, respectivamente[2].

El perfil PK de LPV, coadministrado con RTV, ha demostrado ser similar entre voluntarios sanos y pacientes infectados por el VIH, así como entre pacientes naïve y pretratados a dosis estándar de 400/100 mg (LPV/r) BID vía oral[2,31,32]. También se ha demostrado que la exposición sistémica a LPV en el estado de equilibrio es similar con la dosis de 800/200 mg administrada en una dosis única (QD) o BID en pacientes

naïve[2,32]. Además, no se han apreciado diferencias importantes en la exposición al fármaco ligadas a las diferentes formulaciones: cápsulas blandas, solución oral (ambas en presencia de alimentos) y comprimidos[2,19,21,33].

SITUACIÓN ACTUAL DE LPV/r EN LA TERAPÉUTICA

LPV sigue siendo el único IP coformulado con bajas dosis de RTV disponible. Además, la formulación pediátrica y la solución oral facilitan su utilización en niños y en pacientes con dificultades de deglución y permiten la individualización de las dosis.

En las guías americanas más recientes, LPV/r ha sido desplazado por darunavir/r y atazanavir/r como tercer componente en el inicio del TAR en el paciente naïve[6,7]. La asociación británica de VIH (BHIVA), tampoco lo recomienda en esta situación, proponiendo efavirenz como tercer componente y reservando los IPs para aquellos casos de resistencias a inhibidores de la transcriptasa inversa análogos de nucleósido (ITIANs) y/o no análogos (ITINNs), pacientes con patología neuropsiquiátrica o mujeres gestantes. Sin embargo, en el último Documento de consenso de Gesida y PNS sobre el TAR del adulto, LPV/r se sigue recomendando como IP de primera elección en pacientes naïve al igual que darunavir/r y atazanavir/r.

La experiencia en el uso de LPV/r en terapia de rescate se ha obtenido de los ensayos clínicos realizados en el resto de IPs/r, que utilizan LPV/r como IP de referencia. Tanto tipranavir/r como darunavir/r han demostrado superioridad respecto a LPV/r en el rescate[5-7].

En la actualidad, LPV/r combinado con otros ARTs, continúa siendo una alternativa coste-efectiva tanto en algunos pacientes naïve como en pretratados. Además, ha demostrado un uso seguro en poblaciones especiales, como niños y embarazadas. A pesar de la disponibilidad de nuevos fármacos, la amplia experiencia clínica obtenida

hasta la fecha con LPV/r es de gran valor en terapéutica y garantiza, al menos en un futuro próximo, su continuidad como uno de los IPs de elección en determinadas circunstancias.

ESTRATEGIAS PARA OPTIMIZAR LA TERAPIA

a) Monitorización de niveles de fármacos

Hasta la fecha, aunque la TDM ha demostrado ser útil en la optimización de la terapia ART en determinados pacientes, existe cierta controversia sobre su uso generalizado en la práctica clínica diaria[5-9,34]. De hecho existen guías de consenso donde se recomienda la TDM en situaciones concretas en las que se prevé una alteración en la PK de LPV/r[5-9].

La base fundamental que justifica la TDM es la existencia de una correlación aceptable entre concentraciones plasmáticas y respuesta. No obstante, en ocasiones esta correlación no es fácil de establecer, en especial cuando se trata de terapias combinadas como en el caso del TAR, donde el IP potenciado con RTV (IP/r) normalmente se asocia con dos ITIANs. Este hecho condiciona que los resultados de correlación concentración-respuesta terapéutica varíen según el esquema de TAR y del tipo de paciente[35-38]. En cuanto a toxicidad, aunque no existe una C_{max} consensuada para este fármaco, sí parece demostrada una relación entre niveles plasmáticos elevados de LPV y algunos efectos secundarios[39-41] aunque también se verá influenciada por los factores antes mencionados y la presencia de otros fármacos no ARTs asociados al tratamiento.

A continuación se describen las situaciones en las que actualmente está justificada la TDM de LPV/r.

- Durante el inicio y control del tratamiento

Habitualmente se inicia el tratamiento con LPV/r a dosis estándar de 400/100 mg BID[2], la cual puede no ser adecuada para aquellos pacientes cuyo comportamiento PK se aleje de la media de la población. La determinación de las concentraciones alcanzadas en el paciente al menos una semana después de iniciado el tratamiento, para garantizar el estado de equilibrio, permitirá conocer si son superiores a los límites recomendados de 1 o 4-5,7 mcg/mL en pacientes naïve y pretratados, respectivamente[35,40,42-46]. El resultado obtenido permitirá la detección precoz de concentraciones inadecuadas que pueden dar lugar a la ineficacia del tratamiento, al desarrollo de resistencias o a la presencia de efectos adversos que suelen conducir a una disminución de la satisfacción del paciente con el tratamiento y en consecuencia, a un mayor riesgo de discontinuación de la terapia[47]. Además la estimación individualizada de los parámetros PK de LPV/r permitirá establecer, junto con la evolución clínica, la dosis más adecuada para alcanzar una respuesta óptima en el paciente. Controles sistemáticos periódicos de la respuesta clínica y las concentraciones plasmáticas de LPV/r serán necesarios para asegurar la correcta instauración de la dosis de este fármaco[45,48,49]. De este modo, la TDM ayudaría a verificar si el tratamiento con LPV/r es o no adecuado para el paciente, evitando cambiar precozmente a otras combinaciones de fármacos y así preservar intactas futuras posibilidades de tratamiento.

- En el control de la adherencia al tratamiento

Está ampliamente documentado que la eficacia del TAR está condicionada por la correcta adherencia al tratamiento[50], la cual es difícil de conseguir en un tratamiento crónico complejo que a menudo provoca importantes efectos adversos[51]. Concentraciones plasmáticas de LPV anormalmente bajas y, cuando se dispone de varias concentraciones por paciente, un coeficiente de variación del índice nivel/dosis superior al 100%, podría alertar de la existencia de un problema de adherencia[52].

Aunque la TDM se considera una medida directa de la adherencia, no está exenta de problemas en su detección ya que debido a la corta semivida de eliminación de LPV/r, un cumplimiento adecuado en los días previos al control de la TDM falsearía los resultados de la adherencia, al observarse las concentraciones de equilibrio que realmente alcanzaría el paciente adherente, fenómeno conocido como "adherencia de bata blanca"[53,54]. Por ello, los resultados de la TDM no deben interpretarse aisladamente para evaluar la adherencia, sino junto con otras medidas indirectas, tales como los registros de dispensación o de apertura del envase, cuestionarios SMAQ o entrevistas con el paciente[54,55].

La TDM al individualizar las dosis evitaría concentraciones tóxicas, contribuyendo a mejorar el grado de satisfacción del paciente con el tratamiento e i ndirectamente también la adherencia al mismo[56].

- En la identificación de interacciones con otros fármacos

El paciente VIH frecuentemente está tratado con otros fármacos utilizados para la prevención o tratamiento de infecciones oportunistas, tratamiento sintomático y/o preventivo de los efectos adversos de los ARTs, etc., algunos de los cuales son sustratos, inductores o inhibidores de las enzimas responsables de las mismas vías metabólicas que LPV/r. Así, cuando se asocia al TAR un nuevo fármaco con potencial riesgo de interacción, la TDM proporciona información sobre los niveles de LPV/r antes y después de la asociación, es decir, identifica el sentido e intensidad de la interacción y de acuerdo a el la, permite establecer la dosis más adecuada para obtener concentraciones terapéuticas de este fármaco[45,49,53,57,58]. Por otra parte, LPV/r puede también inhibir el metabolismo de otros fármacos en cuya eliminación esté implicado el CYP3A. En algunos casos, este efecto da lugar a un aumento en las concentraciones plasmáticas de otros fármacos asociados que dan lugar a efectos graves que incluso

pueden amenazar la vida del paciente, contraindicándose su administración conjunta. Esta situación se presenta en fármacos tales como astemizol, terfenadina, midazolam, triazolam, cisaprida, pimozida, amiodarona, alcaloides ergotamínicos, lovastatina, simvastatina, vardenafilo y otros medicamentos a base de plantas que contengan hierba de San Juan (*Hypericum perforatum*)[2].

- En la detección y prevención de la toxicidad

Aunque en general el tratamiento con LPV/r es bien tolerado, con frecuencia aparecen problemas gastrointestinales (diarreas, náuseas y vómitos) y algunas complicaciones metabólicas como la dislipemia, resistencia a la insulina y lipodistrofia[39-41,59]. También se ha descrito una prolongación del intervalo PR y QT, y el aumento del riesgo de hemorragias en pacientes hemofílicos[2]. Considerando que algunos estudios han encontrado una cierta relación entre niveles elevados de LPV/r y presencia de efectos adversos[39-41,49,60], la TDM permitiría detectar situaciones de sobredosificación que podrían resolverse reduciendo adecuadamente la dosis de este medicamento y así evitar un cambio de tratamiento por intolerancia[53]. No obstante, algunos investigadores también sugieren la utilidad de la TDM en pacientes sin manifestaciones aparentes de toxicidad, pero que presentan niveles elevados[45]. Ello es debido a que algunos efectos adversos provocados por el TAR, como las alteraciones metabólicas, suelen aparecer de forma más tardía y en consecuencia podrían ser prevenibles mediante la TDM. En cualquier caso, independientemente de los argumentos que sugieran una reducción de dosis, es importante considerar la concentración diana óptima a alcanzar, la cual varia en pacientes naïve respecto a los pretratados, con valores de C^{ss}_{min} de 1 mcg/mL o de 4 mcg/mL, respectivamente, lo que contribuye a minimizar los riesgos de ineficacia clínica y posible desarrollo de resistencias[35,40,42-46].

- En poblaciones especiales

Durante el embarazo se pueden modificar los procesos de absorción-distribución-metabolismo-excreción (ADME)[45] dando lugar a cambios significativos en las concentraciones plasmáticas de LPV/r. Existe evidencia de que la exposición sistémica a este medicamento se reduce durante el tercer trimestre de gestación[27]. Por ello, se recomienda en mujeres clínicamente controladas antes del embarazo, obtener niveles de LPV/r previos al segundo trimestre de gestación como referencia, los cuales deberán ser mantenidos durante el resto del embarazo mediante los ajustes posológicos necesarios[53].

La población pediátrica es un grupo muy heterogéneo en el que el comportamiento PK de LPV/r difiere de la población adulta. A pesar de las recomendaciones posológicas para niños y adolescentes teniendo en cuenta edad, peso y superficie corporal, no se puede asegurar la misma exposición al fármaco que en adultos[45,53]. Aunque la información disponible acerca de la PK de ARTs en pacientes pediátricos se está incrementando paulatinamente, los datos PK y de eficacia y seguridad de LPV/r en niños menores de 2 años aún son limitados, por lo que no está recomendada su utilización[2]. En este sentido, la TDM cada vez más utilizada en niños y recomendada en las guías PENTA (Pediatric European Network for treatment of AIDS), puede ayudar a conseguir un grado de exposición adecuado en este sector de la población.

En pacientes con pesos extremos también está recomendada la TDM, debido a los potenciales riesgos de toxicidad e ineficacia clínica que puede presentar esta población[15]. Aunque el género por sí mismo no parece afectar el comportamiento PK de LPV/r, el hecho de que con frecuencia las mujeres presenten menor peso corporal puede dar lugar a mayores niveles de estos fármacos cuando reciben la dosis estándar, recomendándose también en ellas la monitorización[45].

Los pacientes con daño hepático son otros candidatos que pueden beneficiarse de la TDM de LPV/r debido al alto grado de metabolismo hepático que experimenta este

medicamento[28,45]. En estos pacientes puede incrementase la exposición sistémica a la fracción libre de LPV/r, debido a una reducción en el metabolismo y en el grado de unión a proteínas plasmáticas[61]. No obstante, ya que habitualmente se determinan las concentraciones plasmáticas de fármaco total, su interpretación puede ser errónea en situaciones en las que la fracción de fármaco libre esté alterada[61]. Aunque hay algoritmos predictivos de las concentraciones de estos fármacos en función del daño hepático, presentan un alto grado de incertidumbre[45]. Además, no se ha establecido la eficacia y seguridad de LPV/r en pacientes con trastornos hepáticos subyacentes significativos[2].

Entre los pacientes infectados por el VIH existen casos de coinfectados con el virus de la hepatitis B o C, con un mayor riesgo de desarrollar fallo hepático secundario al tratamiento con LPV/r[39,62]. En esta situación, la dosis estándar administrada podría resultar potencialmente tóxica, por lo que se recomienda monitorizar los niveles plasmáticos de este IP[47,48,56].

En pacientes con insuficiencia renal, debido a la baja contribución del riñón a la eliminación de LPV/r, no se esperan modificaciones significativas en sus concentraciones plasmáticas[2].

- En regímenes de dosificación fuera de indicación

En esquemas de tratamiento con LPV/r diferentes a los recomendados en las condiciones de autorización, como es la monoterapia, la TDM puede ser una herramienta que asegure concentraciones de LPV/r adecuadas a cada paciente[17,48].

A pesar de la evidencia actual sobre el beneficio de la TDM como herramienta de ayuda en la optimización de la terapia con LPV/r, son necesarias futuras investigaciones que evalúen las prestaciones de esta estrategia. Así, sería importante disponer de mayor

información sobre márgenes terapéuticos de LPV/r que tomen en consideración el tipo de TAR, así como el genotipo del virus y sus parámetros PK en poblaciones específicas de VIH para mejorar la capacidad predictiva de los algoritmos bayesianos. La identificación de polimorfismos genéticos (SNPs) que afecten la PK de LPV/r, revisados en el siguiente apartado, también resultan de interés para su incorporación, como factores de disposición, en el análisis PK[34].

La tabla 1 recoge las recomendaciones de las guías internacionales de consenso sobre el uso de la TDM de LPV/r.

b) Información farmacogenética

La administración de una misma dosis de un fármaco ART a un grupo de pacientes, generalmente da lugar a una elevada variabilidad interindividual en la eficacia y toxicidad del TAR, que puede atribuirse en parte a sus diferencias demográficas y clínicas. Además, entre las causas responsables de esta variabilidad, las variaciones genéticas pueden constituir un factor significativo[63]. Así, se ha demostrado que la presencia de variaciones genéticas en ciertas proteínas implicadas en el metabolismo de LPV/r (CYP3A4, CYP3A5, etc.) y en su transporte (MRP-2, SLCO) pueden influir en su comportamiento PK[15,64-66]. Por otra parte, la presencia de SNPs en aquellos genes que codifican proteínas implicadas en el metabolismo lipídico (APOA5, APOC3, TNF, SREBP1, etc.) afectarán en mayor o menor medida al grado en el que se puede manifestar el síndrome metabólico atribuido a IPs/r[67-70].

Para que la información genética pueda aplicarse en clínica, los genes analizados deben ejercer un efecto dominante, la influencia del genotipo sobre el tratamiento prescrito debe ser significativa y el coste-efectividad de su aplicación debe estar demostrado[9]. Para el caso de LPV/r, aún es preciso establecer claramente la relación entre estos SNPs

y su comportamiento PK, ya que la mayoría de los estudios se han centrado en el efecto de SNPs de un solo gen, mientras que en estos procesos de ADME participan múltiples genes relacionados entre ellos junto con otros factores no genéticos.

Debido al gran interés que está despertando la PG y al número creciente de casos clínicos publicados en los que se demuestra su utilidad en la práctica clínica[10,71], se están realizando diversas investigaciones con los diferentes ARTs para buscar variaciones genéticas que muestren relación significativa con su eficacia y/o toxicidad. Sin embargo, en el caso de los IPs, existen actualmente pocos estudios que evidencien de manera consistente relaciones de este tipo.

En la tabla 2, se muestran los resultados de las variaciones genéticas con posible influencia sobre la PK de LPV/r de los estudios realizados hasta la fecha, indicando el *rs* cuando está disponible y el SNP. Aunque existen indicios, la relación entre los SNPs de CYP3A4, CYP3A5 y SLCO y la PK de LPV/r todavía no está suficientemente documentada, por lo que se precisan más estudios de investigación clínica que la confirmen. Estos hallazgos resultarían de gran utilidad para la prescripción *"a priori"* de las dosis más adecuadas a las características genéticas del paciente y se integrarían con la información PK en los modelos de población[15].

c) Regímenes en monoterapia

Actualmente se intenta conseguir tratamientos con la misma eficacia obtenida hasta ahora, pero con una menor toxicidad y coste. Los IPs/r presentan un favorable perfil PK, una alta barrera genética al desarrollo de resistencias y un elevado cociente inhibitorio, lo que los convierte en excelentes candidatos para su uso en monoterapia[76-79]. Esta estrategia permite a su vez mantener intactas otras opciones terapéuticas[16] y puede estar

asociada con una menor toxicidad a largo plazo, como la lipodistrofia, al retirarse los otros componentes del TAR[80].

La simplificación del tratamiento con dos ITIANs más LPV/r a la monoterapia con este último ha mostrado seguridad y eficacia en un alto porcentaje de pacientes en varios estudios realizados en la última década[81-85]. En caso de fracaso virológico, asociado con frecuencia a problemas puntuales de adherencia y excepcionalmente al desarrollo de resistencias a LPV/r[83], la estrategia de rescate normalmente cursa con el restablecimiento de los ITIANs que el paciente tomaba previamente[81-84].

Con el IP darunavir/r también se han llevado a cabo estudios aleatorizados, tanto en régimen BID como QD[86,87], y por primera vez, se ha demostrado la no inferioridad de monoterapia con un IP/r en régimen QD (darunavir/r) frente a su uso con dos ITIANs[86]. Los datos con atazanavir/r son insuficientes, al igual que con LPV/r en régimen QD[88]. Cabe destacar, que España ha sido pionero junto con otros países en el estudio de esta nueva estrategia de simplificación del TAR a monoterapia con LPV/r, donde se han llevado a cabo algunas de las primeras y más importantes investigaciones hasta el momento[81,83,89].

A pesar de que no existe consenso en las recomendaciones de la monoterapia con IP/r en las diferentes guías internacionales sobre el TAR, está demostrado que una gran proporción de pacientes pueden mantener la supresión viral con LPV/r o darunavir/r en monoterapia. La sociedad europea del sida[8] considera la posibilidad de la monoterapia con LPV/r o darunavir/r como estrategia de simplificación en pacientes en los que exista intolerancia a los ITIANs, que no presenten resistencias a IPs y que hayan tenido la carga viral suprimida al menos en los 6 meses anteriores. La sociedad internacional del sida[7] la recomienda en situaciones muy concretas donde otros fármacos ARTs no pueden utilizarse debido a problemas de toxicidad; sin embargo, el departamento de

salud y servicios humanos estadounidense (DHHS) no recomienda la monoterapia en ninguna situación[6]. En la actualidad se están desarrollando otros importantes estudios sobre monoterapia de IP/r frente a triterapia, cuyos resultados podrían ayudar a los expertos a definir la utilización óptima de esta estrategia[90,91].

d) Administración en régimen QD

En la práctica clínica diaria hay constancia de la administración de algunos IPs en régimen QD en pacientes naïve que iniciaban TAR para facilitar la adherencia, aunque la recomendación autorizada fuera el régimen BID[9]. En el caso de LPV/r, la dosificación QD (800/200 mg) ya ha sido autorizada por la EMA y la FDA. Aunque la dosis total diaria de LPV/r es la misma para los dos regímenes se producen cambios sustanciales en la evolución de las concentraciones de LPV/r, incrementándose significativamente las fluctuaciones entre las C^{ss}_{max} y C^{ss}_{min}. Esta situación puede ser clínicamente relevante, ya que incrementos en las C^{ss}_{max} se relacionan directamente con alteraciones gastrointestinales[17,92] y descensos de las C^{ss}_{min} por debajo de la concentración mínima eficaz pueden comprometer la eficacia del TAR[93], favoreciendo la aparición de resistencias al fármaco[17,92]. No obstante, estudios recientes han mostrado similar eficacia, perfil de tolerancia y desarrollo de resistencias en ambos regímenes de dosificación, tanto en pacientes naïve para el TAR como pretratados[94,95]. Como era de esperar, se ha observado un incremento en la adherencia de aquellos pacientes que toman el régimen QD frente al BID[93-95].

CONCLUSIONES

Algunas guías de consenso siguen recomendando LPV/r como tercer componente del TAR en el paciente naïve; sin embargo, en pacientes con cierto grado de resistencias a

IPs, no está indicado como fármaco de elección. Para optimizar la utilización de LPV/r en terapéutica, existen diferentes estrategias en la práctica clínica, como la TDM. Esta podría ser una herramienta útil para realizar ajustes posológicos de LPV/r en determinadas situaciones donde se modifica su PK, para garantizar concentraciones adecuadas del fármaco. Así mismo, el análisis de la influencia de SNPs de enzimas metabolizadoras y proteínas transportadoras de LPV/r sobre su cinética de disposición, podría ayudar a esclarecer la alta variabilidad interindividual de las concentraciones observadas cuando se administra el fármaco a dosis estándar. Finalmente, el nuevo esquema posológico que utiliza este fármaco en monoterapia, está demostrando una favorable relación riesgo-beneficio, aunque no existe consenso entre las guías internacionales sobre esta indicación para LPV/r.

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TABLA 1. Indicaciones en las principales guías internacionales sobre las estrategias de optimización posológica de lopinavir/ritonavir evaluadas en el estudio

	GESIDA–PNS[5]	DHHS[6]	IAS–UP[7]	EACS[8]	BHIVA[9]
TDM en:					
- Práctica clínica diaria	NR	NR	NR	NR	NR
- Determinadas situaciones*	R	R	R	R	R
Farmacogenética	--	--	--	--	--
Monoterapia	C**	NR	C	C**	--
Régimen QD	C	C	C	C	C

GESIDA – PNS: Grupo de estudio del SIDA-SEIMC – Plan Nacional sobre el SIDA; DHHS: Department of Health and Human Services; IAS – UP: International AIDS Society – USA Panel; EACS: European AIDS Clinical Society; BHIVA: British HIV Association; TDM: monitorización terapéutica de fármacos; R: recomendado; C: considerar; NR: no recomendado; --: Sin información; QD: administración una vez al día; *: Situaciones clínicas que pueden afectar la farmacocinética de lopinavir/ritonavir; **: Solo en pacientes sin fracaso previo a inhibidores de la proteasa, carga viral indetectable al menos durante los 6 meses previos y con historia de síntomas de toxicidad a inhibidores de la transcriptasa inversa análogos de nucleósidos.

TABLA 2. Influencia de polimorfismos genéticos de enzimas y transportadores sobre la farmacocinética de lopinavir/ritonavir

GEN	SNP	refSNP	Consecuencia fenotípica	Referencia bibliográfica
CYP3A4, 3A5, 3A7	ND	rs 6945984	↓ CL_{LPV}	15
CYP3A4	ND	rs 4646437	↔ C_{LPV}	64
CYP3A5	14690A>G (CYP3A5*6)	rs 10264272	↔ C_{LPV}	64,72
	6986A>G (CYP3A5*3)	rs 776746	↔ C_{LPV}	64,72
			↓ C_{LPV} (C_{min})	73
CYP3A7	(promotor alelo 1)	ND	↑ C_{LPV} intracelular	64
CYP2B6	516T>G (CYP2B6*6)	rs 3745274	↔ PK	73
ABCB1 (MDR1)	3435C>T	rs 1045642	↔ C_{LPV} (C_{min})	74,75
			↔ C_{LPV}	64
			↔ PK	73
	2677G>T/A	rs 2032582	↔ C_{LPV} (C_{min})	74
			↔ C_{LPV}	64
			↔ PK	73
	1199G>A	rs 2229109	↔ C_{LPV}	64
	1236C>T	rs 1128503	↔ C_{LPV}	64
ABCC2 (MRP2)	-24C>T	rs 717620	↓ CL_{LPV}	15
			↔ C_{LPV}	64
	4544G>A	rs 8187710	Mayor acumulación de LPV en PBMCs	64
SLCO1B1 (OATP1B1)	463C>A (SLCO1B1*4)	rs 11045819	↑ CL_{LPV}	15
			↔ C_{LPV}	66
	521T>C (SLCO1B1*5)	rs 4149056	↓ CL_{LPV} y ↑ C_{LPV} (C_{min})	15
			↑ C_{LPV} (C_{min})	65,66
			↔ C_{LPV}	64

GEN	SNP	refSNP	Consecuencia fenotípica	Referencia bibliográfica
	g.14076765C>T	rs 4149032	↓ C_{LPV} (C_{min})	65
			↔ PK	15
	ND	rs 11045891	↔ PK	15
	ND	rs 17328763	↔ PK	15
	388A>G	rs 2306283	↔ C_{LPV}	64-66
	11187G>A	rs 4149015	↔ C_{LPV}	64
SLCO1A2 (OATP1A2)	516A>C	rs 11568563	↔ C_{LPV} (C_{min})	65
	38T>C	rs 10841795	↔ C_{LPV} (C_{min})	65
			↔ PK	15
SLCO1B3 (OATP1B3)	334T>G	rs 4149117	↔ C_{LPV} (C_{min})	65
SLCO2B1 (OATP2B1)	ND	rs 1077858	↔ PK	15

SNP: single nucleotide polymorphism; LPV: lopinavir; PBMCs: células de sangre periférica mononucleadas; ND: no disponible; ↓ : disminución de;↑ : incremento de;↔ : sin influencia en; CL: aclaramiento; PK: farmacocinética; C_{LPV}: concentración de lopinavir; C_{min}: concentración mínima.

III. TRABAJO EXPERIMENTAL

POPULATION PHARMACOKINETICS OF LOPINAVIR/RITONAVIR (KALETRA) IN HIV-INFECTED PATIENTS

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Therapeutic Drug Monitoring. 2011;33:573-82.

Population Pharmacokinetics of Lopinavir/Ritonavir (Kaletra) in HIV-Infected Patients

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Background: A relationship between plasma concentrations and viral suppression in patients receiving lopinavir (LPV)/ritonavir (RTV) has been observed. Therefore, it is important to increase our knowledge about factors that determine interpatient variability in LPV pharmacokinetics (PK).

Methods: The study, designed to develop and validate population PK models for LPV and RTV, involved 263 ambulatory patients treated with 400/100 mg of LPV/RTV twice daily. A database of 1110 concentrations of LPV and RTV (647 from a single time-point and 463 from 73 full PK profiles) was available. Concentrations were determined at steady state using high-performance liquid chromatography with ultraviolet detection. PK analysis was performed with NONMEM software. Age, gender, height, total body weight, body mass index, RTV trough concentration (RTC), hepatitis C virus coinfection, total bilirubin, hospital of origin, formulation and concomitant administration of efavirenz (EFV), saquinavir (SQV), atazanavir (ATV), and tenofovir were analyzed as possible covariates influencing LPV/RTV kinetic behavior.

Results: Population models were developed with 954 drug plasma concentrations from 201 patients, and the validation was conducted in the remaining 62 patients (156 concentrations). A 1-compartment model with first-order absorption (including lag-time) and elimination best described the PK. Proportional error models for interindividual and residual variability were used. The final models for the drugs oral clearance (CL/F) were as follows:

$$CL/F_{LPV}(L/h) = 0.216 \cdot BMI \cdot 0.81^{RTC} \cdot 1.25^{EFV} \cdot 0.84^{ATV}$$

$$CL/F_{RTV}(L/h) = 8.00 \cdot 1.34^{SQV} \cdot 1.77^{EFV} \cdot 1.35^{ATV}.$$

The predictive performance of the final population PK models was tested using standardized mean prediction errors, showing values of 0.03 ± 0.74 and 0.05 ± 0.91 for LPV and RTV, and normalized prediction distribution error, confirming the suitability of both models.

Conclusions: These validated models could be implemented in clinical PK software and applied to dose individualization using a Bayesian approach for both drugs.

Key Words: therapeutic drug monitoring, lopinavir, ritonavir, NONMEM, HIV+, patients

(*Ther Drug Monit* 2011;33:573–582)

INTRODUCTION

Lopinavir (LPV) has demonstrated durable antiviral activity in antiretroviral (ARV)-naive and protease inhibitor (PI)-experienced patients.[1–5] In fact, this drug is one of the most widely used PIs for the treatment of human immunodeficiency virus (HIV) infection. However, because of its low oral bioavailability and its extensive metabolism by the CYP3A4 isoenzyme, it needs to be coadministered with ritonavir (RTV) to achieve drug concentrations high enough to inhibit viral replication and allow less frequent dosing.[6–9] Thus, LPV, coformulated with low doses of RTV (Kaletra; Abbott Laboratories, North Chicago, IL), was introduced in 2001 and rapidly displaced other PIs. By mid-2004, it represented approximately 80% of all PIs used in ARV therapy.[10] A combination of 2 PIs in the same dosage formulation decreases total pill burden, thereby possibly increasing patient adherence and acceptance.[11] LPV/RTV was initially developed in soft capsules and a liquid formulation. The liquid is still available, but the capsules have been withdrawn and replaced with tablets (Kaletra-Meltrex) produced by a proprietary melt extrusion-based technology. Drug absorption from the tablet is less dependent on food intake, and exposure is more uniform with the tablet than the capsule formulation.[12]

Relationships between plasma concentrations and efficacy exist for many of the PIs, and in the case of LPV,

Received for publication February 3, 2011; accepted July 12, 2011.
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The authors declare no conflict of interest.
Supported by the Europharma Foundation under an agreement of collaboration between the University of Salamanca and the University Austral of Chile.
Lopinavir and ritonavir, as pure compounds, were kindly provided by Abbott Laboratories. These substances were used both as standards for the analytical technique validation and as standards in all quantitative determinations.
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correlations between exposure and the chance of attaining durable viral suppression in patients receiving LPV/RTV has been observed.[13–19] Target trough concentrations for LPV of 1.0 mg/L have been recommended for PI-naive patients and between 4.0 and 5.7 mg/L for PI-experienced patients.[13,17,18] Various studies have demonstrated that if LPV plasma concentrations are either too high (ie, leading to lipid elevation) or too low (ie, leading to insufficient virological response), especially in treatment-experienced patients, the clinical consequences may be serious.[1,2,17,20–22] Therefore, it is important to increase our knowledge about factors that determine interpatient variability in LPV pharmacokinetics (PK).[18] This situation justifies the use of therapeutic drug monitoring (TDM), which has been widely debated as a tool for optimizing highly active antiretroviral therapy (HAART). In general, this approach seems to contribute to decreased resistance and virological failure, reduced toxicity, and improved overall ARV efficacy. However, further focused clinical trials are needed to more fully define its role.[23] Studies describing the use of TDM in large unselected groups of patients to guide PI dosing have yielded conflicting results.[24–27] Although TDM of PIs may be useful in selected patients, such as pediatric patients, those with renal or hepatic impairment, those who are pregnant, those with potentially concentration-related toxicities, or those with suspected significant drug–drug or drug–food interactions,[19,21] routine use is not supported by current evidence.[23–28]

A prerequisite for the successful application of TDM is the availability of reliable data on the kinetics of ARV drugs in HIV-infected patients. However, there are very few validated LPV/RTV PK models in HIV population using nonlinear mixed effects modeling (NONMEM).[29–33] Furthermore, most of these models have included patients receiving only soft gelatin capsules, a formulation which is no longer available. Consequently, in the present study, LPV and RTV population PK models were developed and validated in adult HIV patients using NONMEM, which allows the estimation of the statistical distribution of compartmental model PK parameters and the identification of covariates that could explain part of the observed variability. Knowledge of population PK of these drugs would allow their implementation in a clinical PK software and its application in dose individualization using a Bayesian approach.[29,30]

METHODS

Patients

Ambulatory patients were recruited from the outpatient pharmacies of 2 Spanish hospitals (Vall d'Hebron in Barcelona and University Hospital of Salamanca in Salamanca) from October 2003 to October 2008. The study included 263 HIV-infected patients (18–70 years old) treated with Kaletra (in soft capsules, liquid formulation, or Meltrex tablets). Most patients were receiving 400/100 mg of LPV/RTV twice daily, doses were adjusted according to PK criteria using Bayesian algorithms, and their concomitant ARV medication consisted of efavirenz (EFV), saquinavir (SQV), atazanavir (ATV), and tenofovir (TFV). A total of 1110 LPV and RTV plasma concentrations from 73 patients with full PK profiles (8–11 plasma concentrations per patient; n = 463) and 190 patients included in a TDM program (1–9 plasma concentrations per patient; n = 647) were available for analysis. This dataset was divided into 2 groups: an index and a validation group. The first one consisting of 415 LPV/RTV plasma concentrations from 67 patients (1 curve per patient) and 539 LPV/RTV concentrations from TDM, randomly selected from the all patients, was used for population PK modeling. The remaining 6 curves (48 concentrations) and 108 plasma concentrations from TDM were used for the model validation.

Table 1 summarizes the demographic, anthropometric, clinical, and treatment characteristics of the patients included in the study. Approval was obtained from the institutional review boards of the hospitals included in the study, and all patients gave written informed consent. They were enrolled in a Pharmaceutical Care Program, and they came to the outpatient pharmacy to pick up their ARV treatment monthly.

The inclusion criteria were as follows: (1) confirmed HIV infection, (2) treatment initiated with unchanged LPV/RTV dose at least 1 month before their inclusion in the study, (3) adherence to the treatment regimen better than 90%, (4) no concomitant use of non-ARV drugs known to interfere with the PK of LPV/RTV,[34] and (5) aged 18 years or older. All patients had normal hepatic and renal function when treatment was started. This information was obtained from the medical history of the patients and the pharmacy files.

Adherence was assessed using pharmacy dispensing records, the Simplified Medication Adherence Questionnaire, and a coefficient of variation (CV) of the mean LPV and RTV plasma concentration to dose ratio in each patient of less than 30%, according to previously observed residual variability.[35,36]

Blood Sampling and Analytical Assay

In the patients with full PK profile available, plasma samples were obtained during 1 dose interval just before

TABLE 1. Demographic, Anthropometric, Clinical, and Treatment Characteristics of the Patients Included in the Study

	Index Set	Validation Set
No. patients (male/female)	201 (112/89)	62 (38/24)
No. LPV and RTV concentrations	954	156
Trough steady state	539	108
From full PK profiles	415	48
Age (yr)	40.3 ± 8.20	42.2 ± 9.30
Height (cm)	170.5 ± 8.10	169.4 ± 8.40
TBW (kg)	68.12 ± 14.62	68.61 ± 12.44
BMI (kg/m^2)	23.49 ± 4.20	23.41 ± 3.40
RTV minimum concentration (mg/L)	0.82 ± 0.67	0.78 ± 0.53
No. patients with HCV	91	33
Total bilirubin (mg/dL)	1.10 ± 0.90	1.07 ± 0.78
Origin (Salamanca/Barcelona)	539/415	108/48
No. concentrations (soft capsules/Meltrex/liquid)	702/234/18	93/56/7
No. EFV/SQV/ATV/TFV concentrations	41/291/136/114	7/45/22/26
LPV mean concentration (mg/L)	7.77 ± 3.58	7.52 ± 3.14
RTV mean concentration (mg/L)	0.89 ± 0.55	0.91 ± 0.51

HCV, hepatitis C virus.

ingestion of the medication and at 1 hour and 2, 3, 4, 6, 8, and 12 hours after observed ingestion of the medication. In patients included in the TDM program, samples were collected at the end of the interval, thus drug levels corresponded to the trough steady state concentrations (C^{ss}_{min}). In these patients, the time when they had last taken an LPV/RTV dose before the sample time was self-reported.

LPV and RTV concentrations were analyzed quantitatively by a validated high-performance liquid chromatography with ultraviolet detection using 600 μL of plasma. Specificity was tested for the 21 compounds most often used by HIV patients.[37] Our analytical laboratories participate in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection [Dutch association for Quality assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT)], and successful results have been obtained. Specificity and accuracy values for the assay are reported in Table 2.

Pharmacokinetic Analysis

Analysis was performed with NONMEM (version VI).[38] The first-order conditional estimation in conjunction with the Laplace approximation was used throughout. The base models describing LPV and RTV PK were of 1 compartment with first-order absorption and elimination (specified to NONMEM by the ADVAN2, TRANS2 routines). The fixed-effect PK parameters directly estimated with this model specification were oral clearance (CL/F), apparent distribution volume (V/F), absorption rate constant (Ka) and absorption lag time (ALAG). Bayesian PK estimates for individual subjects were automatically derived at the end of the first-order conditional estimation population PK modeling run. Proportional error models were used for both PK parameters interindividual variability (IIV) (no variability was estimated for ALAG) and residual variability.

Initial exploration of the relationship between Bayesian individual PK parameters and covariates was performed using both graphic methods and stepwise generalized additive modeling (GAM) implemented in X-pose,[39] a library in S-PLUS (Mathsoft, Inc, Seattle, WA).

Age, gender, height, total body weight (TBW), body mass index (BMI), hepatitis C virus coinfection, total bilirubin, hospital of origin, formulation, and concomitant administration of EFV, SQV, ATV, and TFV were the covariates analyzed. For the LPV model, the RTV trough concentration (RTC) was also analyzed. Quantitative covariates were included in both linear and nonlinear ways, whereas discrete covariates were tested in the models in an exponential way, as binary variables taking 0 or 1 values if absent or present, respectively.

TABLE 2. Characteristics of the Analytical Assay for LPV and RTV

	LPV	RTV
Calibration curve range (mg/L)	1–10	0.5–4.0
Recovery (%)	106.4	99.12
Precision (CV %)	3.5	4.7
Internal quality controls (mg/L)	2.0–6.0–10.0	0.5–2.0–4.0
Limit of quantification (mg/L)	0.50	0.25

The NONMEM objective function value (OFV) is a log-likelihood, and the difference (LLD) between 2 hierarchical models should be at least 3.842 ($df = 1$) for the desired level of significance to be achieved ($\alpha = 0.05$). Other diagnostic criteria for the retention of a covariate included a reduction in unexplained interpatient variability for the associated PK parameters, randomly distributed weighted residuals, a close relationship between the predicted and observed concentrations, and that the 95% confidence interval for fixed-effect coefficients should not include zero (or unity if the covariate is discrete). Thus, the full models obtained were subjected to backward elimination of covariate terms using a more stringent criterion of statistical significance before elimination ($\alpha = 0.01$) (LLD > 6.635; $df = 1$).

Model Validation

To validate the final models for LPV and RTV, their predictive performance was evaluated. The individual parameters estimated with these models for the 2 drugs were used to estimate plasma concentrations (n = 156) in the validation group, obtained at the same time as those actually observed, and both were compared by standard procedures using the mean prediction error and the root-mean-squared error as measures of bias and precision, respectively.[40] Moreover, standardized mean prediction errors (SMPEs), calculated as the mean of individual ratios between prediction errors and SDs of predicted concentrations, were estimated due to the large range of the concentrations and hence the residuals.[41] To evaluate the proposed models, normalized prediction distribution error (NPDE) was computed as described by Brendel et al[42] from 1000 replicates for each observed data point of the validation set, which were generated with NONMEM. Wilcoxon signed rank test and Fisher test were used to test whether the mean and the variance were significantly different from 0 and 1, respectively, and the Kolmogorov–Smirnov test was used to probe if NPDE followed a N[0,1] distribution.[43] Complementary statistical analyses were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 14.0.1 (SPSS, Inc, Chicago, IL).[44]

RESULTS

For LPV and RTV data concentration adjustments, different PK models were investigated, including 2-compartment models and zero-order absorption. However, they proved to be less satisfactory than 1-compartment model with first-order absorption and elimination. Moreover, the addition of an ALAG for both drugs significantly improved the fit ($P < 0.005$).

In the LPV basic model, without covariates, the estimated values for CL/F, V/F, Ka, and ALAG were 3.73 L/h, 115 L, 0.786 per hour, and 0.675 hours, respectively, and the observed IIV (except for ALAG, which was not estimated) was high with values ranging from 37.71% to 172%. For RTV, values of 8.8 L/h, 321 L, 2.16 per hour, and 2.33 hours were obtained for CL/F, V/F, Ka, and ALAG, respectively, and their IIV was also high with values from 48.27% to 96.2%.

Figure 1 shows some significant relationships found between post hoc Bayesian CL/F_{LPV} and different covariates

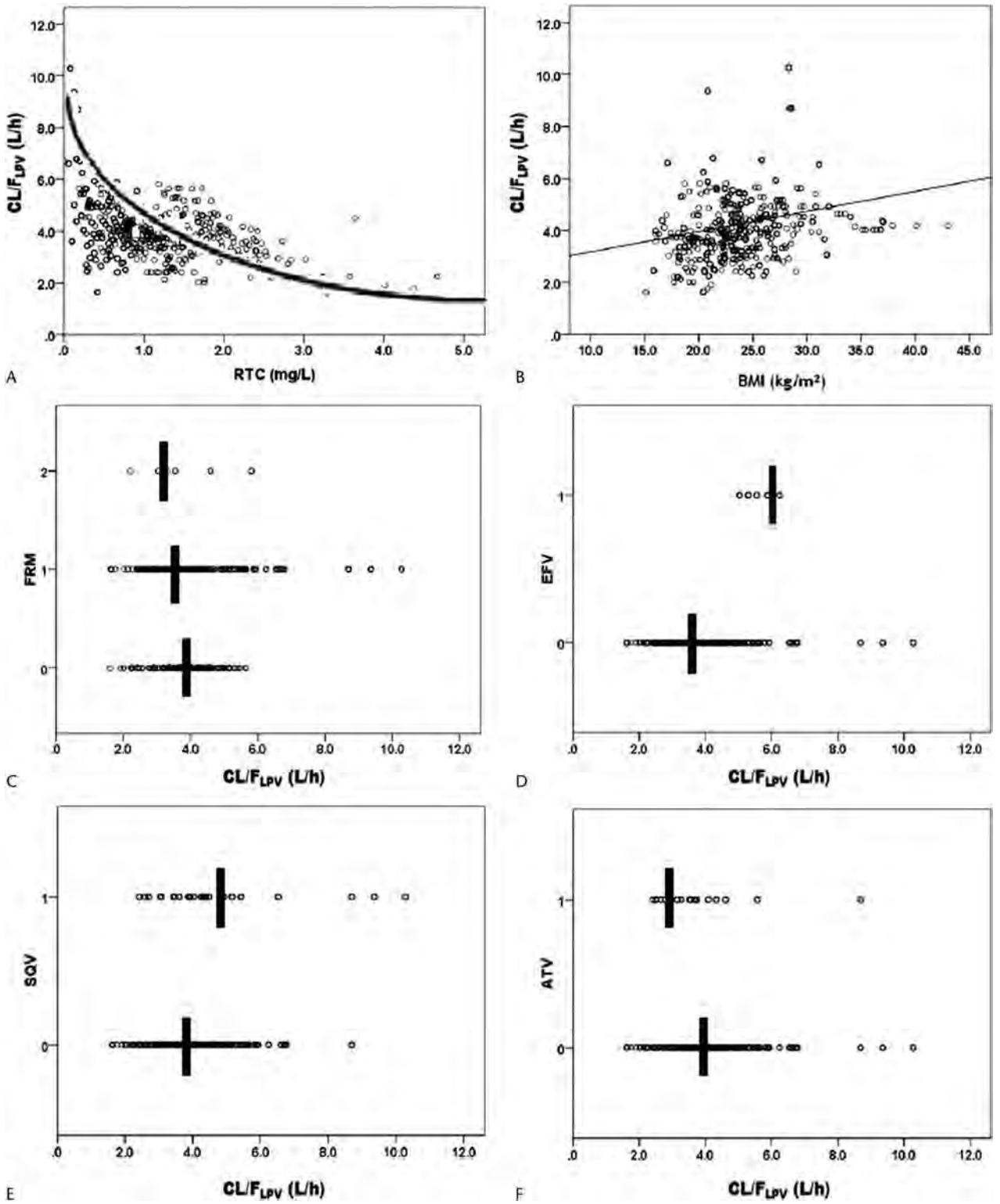

FIGURE 1. Relationships between post hoc Bayesian LPV oral clearance (CL/F_{LPV}) and (A) RTC, (B) BMI, (C) formulation (FRM: 0, Meltrex tablets; 1, soft capsules; 2, liquid formulation), (D) concomitant administration (0, absence; 1, presence) of EFV, (E) concomitant administration (0, absence; 1, presence) of SQV, and (F) concomitant administration (0, absence; 1, presence) of ATV.

investigated using X-pose, which showed statistical influence on the PK parameter estimated.

To assess the potential differences in PK parameters between patients from the 2 Spanish hospitals, Barcelona and Salamanca, a statistical comparison obtained with the basic model in both the populations was performed. Because no discrepancies were observed between the sets, it was not necessary to split the patients according to their origin. Furthermore, no influence of different formulations (soft capsules, Meltrex tablets, and liquid) was observed on the PK profiles (Fig. 1C).

None of the PK parameters assessed for LPV was found to be significantly different for women than for men, suggesting a lack of gender difference in the PK of this drug. In relation to the covariates reflecting body size, GAM revealed that BMI is the best predictor for CL/F_{LPV} (Fig. 1B). To verify this, models with TBW and BMI were assessed. The best data fit was with the latter (OFV: 2053.339 versus 2031.739; $CV_{CL/F}$: 34.6% versus 36.1%), but neither TBW nor BMI affected V/F or Ka significantly.

The magnitude of the RTC seems to affect CL/F_{LPV}, the latter decreasing with increasing RTC (Fig. 1A). This boosting effect is well known and is usually related to the area under the curve (AUC) of RTV (AUC_{RTV}).[8] The strong correlation found between AUC_{RTV} and RTC (Fig. 2) in patients with full PK profiles (n = 68; r^2 = 0.881) justifies the inclusion of RTC instead AUC in the model.

The influence of coadministration with EFV, SQV, ATV, and TFV on CL/F_{LPV} suggested that only the presence of EFV and ATV significantly influences this PK parameter (Figs. 1D, F). Thus, BMI, RTC, EFV and ATV were the covariates with statistical influence on CL/F_{LPV} as they provided a difference in objective function value of 355.992 with respect to the basic model ($P < 0.05$) and a significant improvement of the goodness of fit of the data, as can be seen in Figure 3, which shows the predicted LPV plasma concentration versus population observed concentration from the basic and the final model. Consequently, the following equation describes the final regression model for CL/F_{LPV}:

$$CL/F_{LPV}(L/h) = \theta_1 \cdot BMI \cdot \theta_5^{RTC} \cdot \theta_6^{EFV} \cdot \theta_7^{ATV},$$

where EFV and ATV are "1" for individuals treated with these drugs in their HAART and "0" for all others. The values of population parameters of the LPV final model are summarized in Table 3. Estimated standard errors (SEs) were less than 24% and 53% for fixed and random parameters, respectively.

In relation to RTV, concomitant treatment with EFV, ATV, and SQV significantly influenced its oral clearance, thus the structure of the regression equation for this PK parameter in the final model was as follows:

$$CL/F_{RTV}(L/h) = \theta_1 \cdot \theta_5^{SQV} \cdot \theta_6^{EFV} \cdot \theta_7^{ATV},$$

where SQV, EFV, and ATV are 1 for individuals using this drug in their HAART and 0 for all others. The remaining RTV PK parameters, Ka, V/F, and ALAG, were not statistically influenced by any of the covariates analyzed in this study. The values of fixed and random parameters estimated for the final PK model of RTV (Table 4) showed SE less than 22% and 40%, respectively.

Plots of LPV and RTV population predicted versus measured concentrations in the validation set of patients are shown in Figure 4. Mean prediction error ± SD and SMPE ± SD were −0.01 ± 2.25 mg/L and 0.03 ± 0.74, respectively, for LPV, whereas they were found to be 0.01 ± 0.41 mg/L and 0.05 ± 0.91, respectively, for RTV. Figure 5 shows the quantile–quantile plot of the LPV and RTV NPDE distribution versus the uniform distribution and NPDE calculated values versus predicted concentrations. Mean and variance of LPV NPDE were found to be 0.00443 (SE = 0.13) ± 0.7905 (SE = 0.15) and mean and variance of RTV NPDE were found to be 0.04842 (SE = 0.14) ± 0.8878 (SE = 0.17). The probabilities found by Wilcoxon signed rank test (0.0734 for LPV and 0.916 for RTV) and Fisher test (0.0642 for LPV and 0.186 for RTV) were greater than 0.05, thus we can accept both null hypotheses (mean = 0 and variance = 1) for both the drugs. From Figure 5, the assumption of N[0,1] distributions seems reasonable because deviations from the identity lines show minimal departures from the expected distribution. In fact, the Kolmogorov–Smirnov test showed that NPDE follow a N[0,1] distribution (P = 0.412 and 0.999 for LPV and RTV, respectively), and hence, the hypothesis tested can be accepted, and the proposed models can be considered adequate.

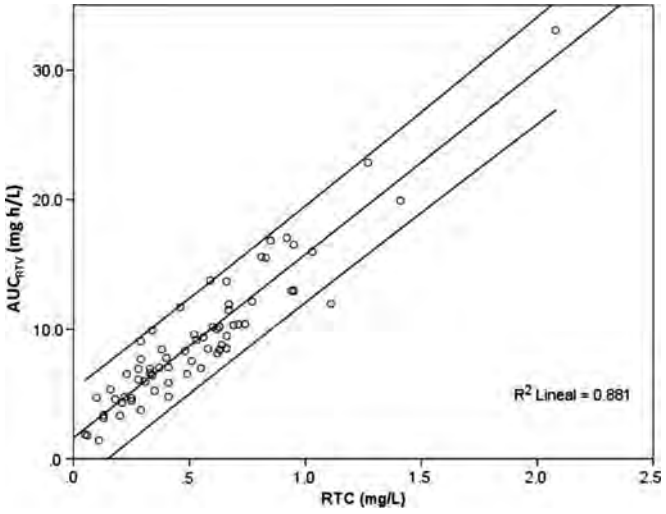

FIGURE 2. Linear relationship between the AUC_{RTV} and RTC.

DISCUSSION

Despite the widespread use of LPV/RTV, Kaletra, available data on their PK using mixed effects population approach in clinical practice are still few,[29–33] and the Meltrex formulation was only included in some of these studies.[31,32] In this study, population models describing the PK of RTV and LPV have been developed and validated in HIV-infected Spanish patients treated with Kaletra.

For other PIs frequently used at the moment, such as ATV or darunavir, there are very few data on their population

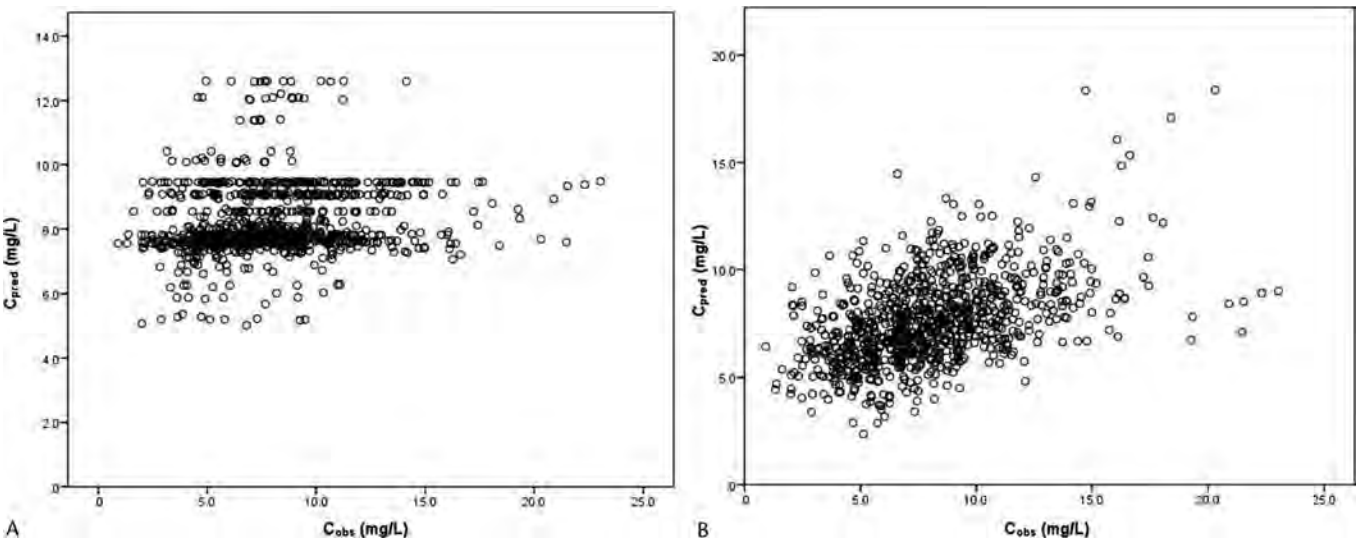

FIGURE 3. Predicted LPV plasma concentrations (C_{pred}) versus population observed concentrations (C_{obs}) from (A) the basic and (B) the final models.

PK in HIV-infected patients.[45–48] However, for EFV, several population PK models have been published.[49–53]

The kinetic profiles of LPV and RTV were adequately described by a structural model consisting of a 1-compartment model with first-order absorption (including a lag time) and elimination. These PK models were similar to those described by other authors, but some differences in the PK parameter values were found.[29–33]

In agreement with a previous report,[54] our results showed a lack of effect of the different formulation administered, soft capsules, Meltrex, and liquid, on the PK parameters analyzed. LPV plasma concentration versus time curves were characterized by an irregular absorption phase showing double peaks in some samples, in accordance with other authors.[18,55] The mean Ka value obtained for LPV (1.9 ± 1.50 per hour) was different from those observed in other studies (0.35–0.85 per hour), although the irregular profiles of the absorption phase and the high IIV (78.99%) could be responsible for this discrepancy.[30–32,55,56] However, the mean value for ALAG (1.78 hours) was similar to that estimated by other authors (0.71–2.35 hours).[30–32,55,56]

Although most population PK studies do not include body size–related covariates in CL/F_{LPV},[29,30,55] some of them found an influence of BMI or TBW.[18,31,57,58] In our study, TBW and BMI were associated with CL/F_{LPV} in GAM analysis. However, BMI instead of TBW was included in the final model because patient BMI correlated better than TBW (difference in objective function value = 21.60).

TBW significantly decreased OFV of V/F_{LPV}. However, it did not really improve the model fit as an unacceptable SE was reported for the remaining PK parameters. Therefore, TBW was not included in the final model. This result is in agreement with most PK studies, which also did not include this covariate on V/F_{LPV}.[29,30,55]

The influence of gender on LPV PK has become a subject of intense clinical investigation.[29,30,55,58–61] Most

TABLE 3. Parameter Estimates and Estimated SE for LPV Final Population Model*

Parameter	Estimate	SE (%)
θ_1 (L/h)	0.216	4.38
θ_2 (L)	130	10.1
θ_3 (h^{-1})	1.90	19.4
θ_4 (h)	1.78	4.30
θ_5	0.81	11.2
θ_6	1.25	23.3
θ_7	0.84	21.2
$CV_{CL/F\ LPV}$ (%)	27.6	14.5
$CV_{V/F\ LPV}$ (%)	52.3	52.2
CV_{Ka} (%)	78.9	29.1
σ (%)	19.0	8.14

*Final model: $CL/F_{LPV} = \theta_1 \cdot BMI \cdot \theta_5^{RTC} \cdot \theta_6^{EFV} \cdot \theta_7^{ATV}$; $V/F_{LPV} = \theta_2$; $Ka_{LPV} = \theta_3$; and $ALAG_{LPV} = \theta_4$.

TABLE 4. Parameter Estimates and Estimated SE for RTV Final Population Model*

Parameter	Estimate	SE (%)
θ_1 (L/h)	8.00	4.24
θ_2 (L)	275	16.7
θ_3 (h^{-1})	1.57	21.8
θ_4 (h)	1.96	0.75
θ_5	1.34	10.9
θ_6	1.77	19.1
θ_7	1.35	8.30
$CV_{CL/F\ RTV}$ (%)	43.5	15.4
$CV_{V/F\ RTV}$ (%)	88.3	19.0
CV_{Ka} (%)	63.1	39.5
σ (%)	31.8	10.4

*Final model: $CL/F_{RTV} = \theta_1 \cdot \theta_5^{SQV} \cdot \theta_6^{EFV} \cdot \theta_7^{ATV}$; $V/F_{RTV} = \theta_2$; $Ka_{RTV} = \theta_3$; and $ALAG_{RTV} = \theta_4$.

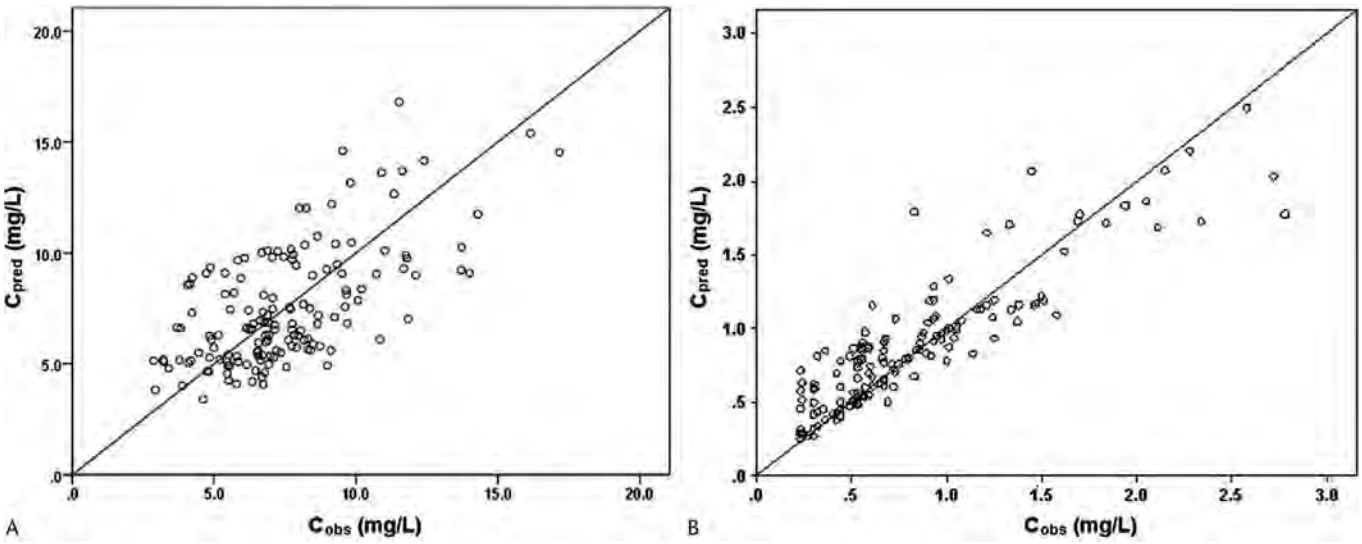

FIGURE 4. Predicted (A) LPV and (B) RTV plasma concentrations (C_{pred}) versus observed concentrations (C_{obs}) from the final model in the validation set.

researchers failed to demonstrate gender-related differences in the LPV PK parameters evaluated,[29,30,55,58–60] except 1 report with statistically significant higher concentrations of LPV in women than in men, but the differences in their body weights (lower in women than in men) could have led to this finding.[61] In our analysis, gender did not improve the fit of the data and it could not be introduced in the model, which justifies the current practice of using the same LPV/RTV dosing schedule for male and female HIV-infected patients.

As can be expected, in our study AUC_{RTV} was significantly associated with CL/F_{LPV} when full PK profiles were available.[8,62] The close relationship observed between AUC_{RTV} and RTC (Fig. 2), which had not been demonstrated previously, allows the use of RTC instead of AUC_{RTV} as a predictive covariate of CL/F_{LPV}. In fact, RTC was identified as the most important covariate influencing CL/F_{LPV} as it explained most of the IIV (22.25%) found for this PK parameter. This result can be seen as an interesting approach in a clinical setting because it would permit the prediction of the RTV boosting effect on LPV from TDM (samples at the end of the dosage interval). This result avoids the exhaustive sampling schedule required to perform AUC_{RTV} estimation.

It is foreseeable from our results and those of other studies that drug interactions can significantly contribute to IIV.[63] There are some studies evaluating the influence of EFV on LPV/RTV PK.[64–67] In this study, an increment of 25% in CL/F_{LPV} value was observed when EFV was coadministrated. This result is in agreement with previous studies,[64,66,67] except 1 study, in which this relationship was not found.[65] Because this effect is widely demonstrated in the literature, an increment of LPV/RTV dose (600/150 mg twice daily) is recommended by international guidelines.[19]

The possibility of a potential drug–drug interaction between LPV and ATV has also been reported previously.[57,62] Our results showed that CL/F_{LPV} is reduced by a factor of 0.84 when this drug is coadministered, leading to an increased LPV exposure. This interaction seems to have a relevant quantitative impact, but international guidelines do not recommend LPV/RTV dose reductions. So, in those centers where TDM is available, it would be possible to individualize the dose in this situation, always with strict clinical control.[68]

The other ARVs analyzed were apparently without effect on LPV elimination. Coadministration of TFV showed a slightly lower LPV/RTV exposure, a trend also observed by others[59,69–72] but not considered by some.[29,30,32] The fact that this covariate did not fulfill the required statistical criteria did not allow its inclusion in the final model.

As has also been reported previously, SQV did not show a significant influence on CL/F_{LPV}.[73] However, this drug seems to increase CL/F_{RTV}, diminishing its booster effect on CL/F_{LPV}. In fact, as can be seen in Figure 1, CL/F_{LPV} tends to be higher when SQV is administrated (Fig. 1).

As a result of different approaches used in PK characterization, it is difficult to compare PK parameters with some of those outlined in the literature, but, in general, the estimated PK parameters of LPV were consistent with values reported from controlled clinical trials.[29,30,32,55,56] Thus, our estimation for CL/F_{LPV} fell within the wide range of mean values reported in the literature (4.23–11 L/h). However, the estimated V/F (130 L) was higher than that found in previous studies (70.8–91.6 L).[29,32]

Estimated LPV PK parameters showed high intersubject variability that could not be explained by the covariates included in the final model. The magnitude of this IIV observed in CL/F, V/F, and Ka were 27.57%, 52.34%, and 78.99%, respectively. The inclusion of covariates only on CL/F justifies its low variability compared with the other PK parameters. The magnitude of this variability was similar to that found in other studies and justifies the TDM of these drugs.[29,55]

The magnitude of residual variability found was relatively low (19.05%) despite the factors involved:

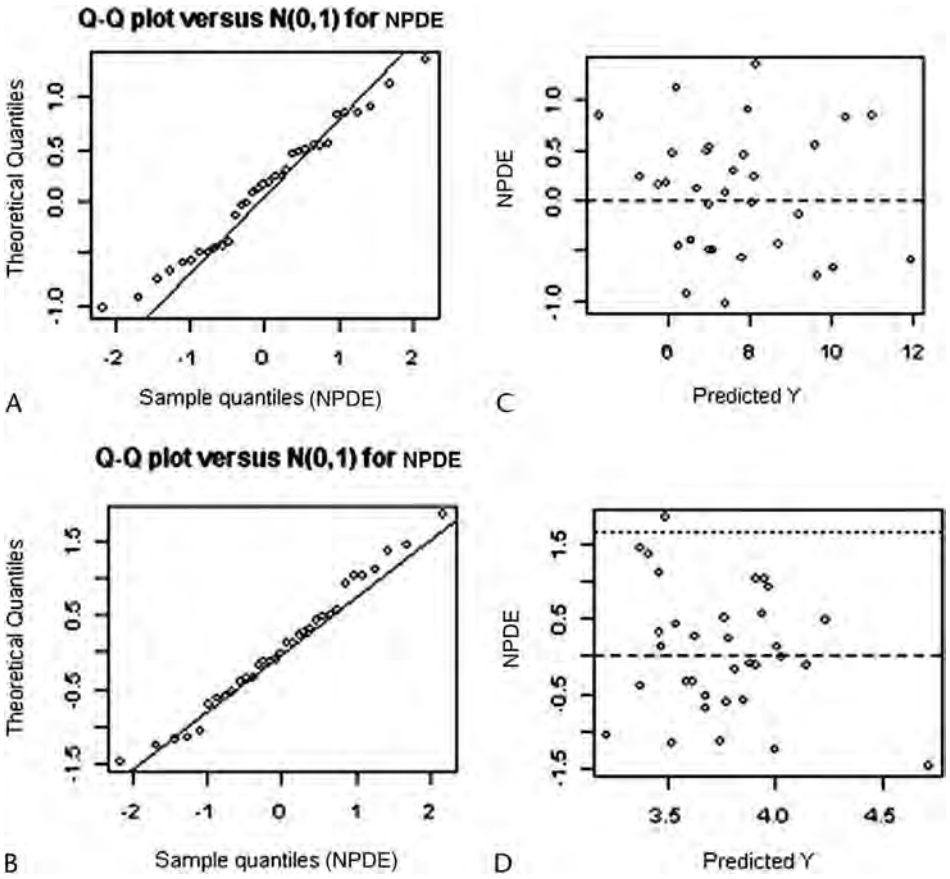

FIGURE 5. LPV (A) and RTV (B) quantile–quantile plots of the NPDE versus the uniform distribution, and LPV (C) and RTV (D) NPDE values versus predicted concentrations.

intraindividual PK and assay variability, food effects, concomitant use of prescription and herbal medicines, medication timing, and treatment adherence. This low value is acceptable and useful for providing a direct assessment of the range of concentrations expected within an individual and allows the use of this criterion as a direct measure of adherence. Instead of considering <30% as intraindividual CV of the mean LPV plasma concentration/dose ratio, 20% could now be used.

Although the population PK model obtained for RTV reveals the influence of some of the covariates analyzed, it is of secondary importance with respect to the LPV model due to RTV acting as a booster, instead of an ARV agent in this association. This booster effect is included in the LPV model being quantified by the relationship between RTC and CL/F_{LPV}.

It has been reported that dose may be a determinant of the PK behavior of RTV, probably because of saturable hepatic metabolism or dose-dependent first-pass loss through intestinal CYP3A4, as reported by Hsu et al.[74] Thus, the values of PK parameters obtained in our study can only be compared with those estimated for a dose of 100 mg twice daily. For this RTV dose, the estimated PK parameters were comparable with previously reported values, except for V/F, which was higher than that found in other studies.[5,8,33]

RTV clearance was significantly influenced by concomitant EFV, ATV, and SQV treatment, which were included in the final population model as categorical covariates (0/1). This effect has been quantified, showing an increase of 77%, 35%, and 34% on CL/F_{RTV} when EFV, ATV, and SQV are present in the HAART, respectively. The same tendency with respect to SQV has been observed by Ribera et al,[58] although the influence was not statistically significant. However, Kappelhoff et al[33] and Cameron et al[75] did not find any influence of this drug on RTV PK. In addition, for EFV, the increase in the CL/F_{RTV} when both drugs are coadministered had been described previously as showing a moderate interaction.[67] However, in the case of ATV, different results have been found. Although some authors have observed a decrease on CL/F_{RTV}, which increases AUC_{RTV}, in a range from 26% to 70% in the presence of ATV,[57,62] no influence of this drug has been documented by others.[76]

The final models obtained for LPV and RTV produced an adequate fit to the data, as confirmed by the validation analysis using SMPE and NPDE.

The magnitude of the IIV in LPV/RTV PK involves a broad range of expected plasma concentrations for a fixed-dose regimen, which could justify TDM of these drugs in HIV-infected patients. Thus, TDM may play an important role in the detection of either suboptimal or potentially toxic concentrations of these PIs, which is an important factor in the success of HAART and management of HIV. In this sense, having LPV and RTV population PK models in this group of patients is essential for predicting individual PK behavior using the Bayesian approach.

CONCLUSIONS

A population model to predict the PK of LPV/RTV has been developed and validated in HIV-infected patients for both the drugs. BMI and concomitant treatment with EFV, ATV, and SQV must be considered as covariates affecting PK behavior of LPV and RTV. Both LPV and RTV interact, and the magnitude of the RTC seems to affect LPV elimination significantly. The population models proposed adequately describe LPV and RTV PK and can be used in Kaletra dose individualization in TDM using the Bayesian approach.

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PHARMACOGENETIC ANALYSIS OF SNPs IN CANDIDATE GENES INVOLVED IN RESPONSE AND EFFICACY DURING THE LOPINAVIR/RITONAVIR THERAPY: NEW ASSOCIATIONS OF SNPs IN DRD3 GENE

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INTRODUCTION

Understanding why some individuals establish and maintain effective control of HIV-1 during the combined antiretroviral therapy (cART), and other people do not, is one of the goals in the clinical management of the HIV-infection (Fellay 2007). Despite the known benefit and the experienced use of lopinavir/ritonavir (LPV/r), a huge interindividual variability (IIV) in plasma concentrations, response and development of adverse events during the treatment with this drug has been observed (Moltó 2006, Van der Leur 2006). In this sense, the treatment with standard doses of 400/100 mg of LPV/r twice daily could lead to non-optimal plasma trough concentrations in some patients, with the emergence of drug resistance and absence of immunovirological response (Johnson 2006, Voigt 2004) or toxicity in others (Gutiérrez 2003, González 2004).

There are many causes that contribute to this interpatient variability, such as demographic, clinical, environmental and host genetic factors (Owen 2008). They could affect the expression and functional activity of many proteins involved in the absorption-distribution-metabolism-excretion (ADME) process, in the immune recovery or in the adverse effects related to the treatment with LPV/r (Aspiroz 2011, Moltó 2008, Lubomirov 2010, Owen 2008, Fellay 2002, Pacenti 2006, Arnedo 2007). Additionally, the natural course of the HIV causes a production decrease and a destruction increase of the CD_{4+} cells, which also contributes to the unpredictable response among HIV-infected individuals (Faure 2000).

The study of single nucleotide polymorphisms (SNPs) in genes encoding for the cytochrome P450 (CYP) enzymes (Kumar 1999), drug transporters (Agarwal 2007, Hartkorn 2010) and some receptors involved in ADME process of LPV/r, as well as

elements implicated in the regulation of the expression of these proteins (Kliewer 2003), is being of critical importance to better characterize ADME process and the phenotype of the HIV-infected patients. However, the impact of SNPs in CYP3A4, CYP3A5 and ATP binding cassette (ABC) type B1 (ABCB1) genes on LPV/r kinetics still remains controversial (Lubomirov 2010, E lens 2009, Estrela 2010, Gupta 2008, M a 2007, Winzer 2003). Maybe the potent boosting effect of ritonavir (RTV) could hide the influence of these genetic variations (Owen 2008).

As other protease inhibitors (PIs), LPV/r is also a substrate for other efflux transporters of the ABC family, like ABCC1 and ABCC2, and for the organic anion transporting polypeptides (OATP), OATP1B1, OATP1B3 and OATP1A2, which are membrane influx transport proteins encoded for by t he SLCO genes (Agarwal 2007, Hartkorn 2010). These proteins are involved in disposition and apparent clearance of PIs, so current pharmacogenetic (PG) studies have focused on SNPs in ABC family and SLCO genes in order to clarify part of the unexplained PK variability (Owen2008, Lubomirov 2010, Elens 2009, G upta 2008, M a 2007, W inzer 2003, Hartkorn 2010, Kohlrausch 2010).

On the other hand, the metabolic syndrome is one of the most dramatic adverse effects attributed to the HIV and the cART due to its potential cardiovascular risk factor and the social impact of the lipodystrophy (Bozzette 2003, F riis-Moller 2007, Carr 1998). Because it is not developed in all of the patients or with the same severity, it has been postulated a host genetic predisposition (Fauvel 2001, Tarr 2005, Foulkes 2006, Maher 2002). Moreover, dyslipidemias have been specially described among patients taking RTV-boosted PIs regimens (Friis 2007, Fontas 2004).

Several studies have stabilized relationships between genetic variations within apolipoproteins (APO) genes and plasma lipid levels (Guardiola 2006, Arnedo2007, Fauvel 2001, Tarr 2005, Foulkes 2006); however, inconclusive data exist about the role of cholesteryl ester transfer protein (CETP), the sterol regulatory ester binding protein type 1 (SREBP-1), leptin (LEP) genes and tumor necrosis factor (TNF) as well as other immunomodulators, which are also involved in lipoprotein and adipocyte metabolism (Tarr 2007, Miserez 2001, Sandhofer 2008, Pacenti 2006, Nolan2003). Thus, exist few researches analyzing the impact of SNPs in candidate genes on the risk of developing PI-related dyslipidemia (Sandhofer 2008, Steinle 2004, Guettier 2005). In this sense, identifying HIV-infected individuals who are at increased risk of developing toxicity could be of relevant significance in clinical practice to choose therapeutic choices that maintain viral suppression while reducing adverse events.

This is one of the largest genetic association studies which involves the genotyping of 290 SNPs concerning candidate genes in 109 HIV-infected individuals. The identification and confirmation of those showing significant influence on IIV of the PK parameters of LPV/r, immunovirological response or toxicity derived from the treatment with this drug could be helpful as a tool to optimize the LPV/r-based cART (Van der Leur 2006, Gutiérrez 2003, Masquelier 2002).

MATERIAL AND METHODS

Study Population and Design

This study included HIV-infected patients recruited from the outpatient pharmacy of the University Hospital of Salamanca (Spain) from January 2006 to January 2011. All

patients received the standard dose of LPV/r, 400/100 mg twice daily, as component of their cART.

The inclusion criteria were as follows: confirmed HIV infection, treatment initiated with standard LPV/r dose at least one month before their inclusion in the study, aged 18 years or older, adherence to the treatment regimen better than 90% and no concomitant use of non antiretroviral (ARV) drugs known to interfere with the PK of LPV/r.

The patients were enrolled in a Pharmaceutical Care Program where therapeutic drug monitoring (TDM) of ARVs was carried out. The plasma samples for the LPV/r assay were drawn periodically at 3 to 6 month intervals on follow-up visits to the hospital, along with viral and biochemical tests. One of these samples was used to the genetic analysis. Other individual information was carefully recorded from the medical history and at the time of collecting the blood samples, and included: dose history, sampling time, time of last dose before sampling, age, weight, height, gender, race, hepatitis C virus (HCV) coinfection, concomitant treatment with tenofovir (TFV), biochemical data (total bilirubine levels [TB], lipid profile, CD_{4+} cell count), HIV viral load (VL) and the presence of diarrhea during the treatment with LPV/r.

Adherence was assessed using pharmacy dispensing records, because the patients came to the outpatient pharmacy to pick up their treatment monthly, the Simplified Medication Adherence Questionnaire and a variation coefficient (CV) of the mean LPV/r plasma concentration/dose ratio in each patient of less than 30%, according to previously observed residual variability (Knobel 2002, Csajka 2003).

Approval was obtained from the institutional review boards of the University Hospital of Salamanca and all patients gave written informed consent.

Drug Assay

Blood samples (5 mL) were collected and the plasma was isolated by centrifugation at 3000 *g*. Then, the samples were stored at -20°C (following virus inactivation in a water bath at 60°C for 60 min) until analysis. Plasma samples were usually collected at the end of the interval, thus LPV/r levels corresponded to the trough steady-state concentrations (unchanged dose at least one month before).

LPV/r concentrations were analyzed quantitatively by high-performance liquid chromatography with ultraviolet detection system (Waters, Milford, USA) at 215 nm after previous solid phase extraction on the GX-271 ASPEC (Gilson, Villiers le Bel, France) (Colombo 2004). This method was validated over the 2.0 to 10 mg/L range using 600 µL of plasma. The recovery of LPV/r from human plasma was 106.4%. Within and between-day precisions, expressed as CV, were always < 3.5% for all the internal quality controls (2.0, 6.0 and 10.0 mg/L). The limit of quantification was 0.5 mg/L. Specificity was tested for the 21 compounds most often used in HIV patients.

Our analytical laboratory participates in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection (Dutch association for Quality assessment in Therapeutic Drug Monitoring and Clinical Toxicology [KKGT]), and successful results have been obtained.

Estimates of Pharmacokinetic (PK) Parameters

The PK of LPV/r was characterized assuming a one-compartment kinetic model with first-order absorption and elimination, using the nonlinear mixed effect modeling program (NONMEM® version VI; double precision, level 2.0) (Beal 1989). The estimated PK parameters according to a previous PK population study by our group were the apparent oral clearance (CL/F), apparent distribution volume (V/F), absorption rate constant and the absorption lag time, 4.26 L/h ($CV_{CL/F}$ = 27.6%), 130 L ($CV_{V/F}$ = 52.3%), 1.9 h^{-1} (CV_{Ka} = 78.9%) and 1.78 h, respectively. These parameters were implemented in clinical PK software (PKS® software, Abbott-Diagnostic, Chicago, USA) and applied to estimate individual CL/F, V/F, the maximum, mean and minimum steady-state plasma concentration (C^{ss}_{max}, C^{ss}_{mean} and C^{ss}_{min}, respectively) for each patient, using Bayesian algorithms from the LPV/r plasma concentration data. These individual PK parameters and concentration data estimated were used for statistical analysis.

Efficacy and Toxicity Data

In the routine plasma samples for the LPV/r assay, lymphocyte CD_{4+} cell count and VL were determined as indicative of immunovirologic efficacy; the lipid levels (triglycerides [TG], total cholesterol [TC], low-density lipoprotein cholesterol [cLDL], high-density lipoprotein cholesterol [cHDL] and cLDL/cHDL coefficient) were also determined in order to evaluate lipid related disorders; and TB was analyzed as hepatic function indicator. From the clinical history, information concerning the presence of diarrhoea was also collected to evaluate the gastrointestinal toxicity associated to this treatment.

SNP Selection and Genotype Analysis

The SNPs were selected in order to carry out an association genetic study from candidate genes. These genes were previously reported by other researches in different ethnic groups in public databases as including SNPs with identified or presumed functions in genes encoding for enzymes and transporters of PIs (LPV and RTV mainly) or lipid metabolism pathways. Different databases consulted were: CYP alleles: www.cypalleles.ki.se, for the metabolizing enzymes, and NCBI: www.ncbi.nlm.nih.gov/SNP/ and SYSNPs: http://www.sysnps.org, for the other genes. SNPs chosen were either functional SNPs (based on pot ential protein changes non synonymous), or in linkage disequilibrium with a functional one, and some of them were in the promoter region of the genes studied. From NCBI and SYSNPs databases, were selected the SNPs with a high (> 20%) minor allele frequency (MAF) in Europeans according to the EMSAMBL database and those tagSNPs which were representative of some genes.

A total of 290 SNPs were chosen using the above criteria and included in the analysis. SNPs investigated and their frequencies in the patients included in this study are shown in Table 1.

Genomic DNA was isolated automatically from 5 mL whole human blood with magnetic bead technology using the Chemagic Magnetic Separation Module I and the Chemagic DNA kit, according to the manufacturer's instructions (Chemagen AG, Baesweiler, Germany).

Genotyping was performed using a MassArray platform provided by Sequenom. This method involves multiplex PCR amplification of up to 22 SNPs. Excess nucleotides are removed by treatment with shrimp alkaline phosphatase, and multiplex primer extension is performed using a mix of deoxy- and dideoxynucleotides, so that products of different masses are obtained for each allele of each SNP. Resulting products are resolved by mass spectrometry (MALDI-TOF). The analysis of the SNPs in metabolizing enzymes and transporters genes was conducted from some preestabilished designs in the MALDI-TOF platform at the Spanish National Genotyping Center (CeGen) in Santiago de Compostela (Spain). For SNPs in selected genes to evaluate the toxicity new designs were used in MALDI-TOF technique. Difficult genotyping rs361525, rs1800629, rs3093662, rs5128, rs4520, rs2070669, rs12718464, rs2070665, rs5072, rs429358, rs7412, rs3745274 rs8192709 rs3211371, rs662799, rs3135506, rs2075291, rs619054 and rs651821 SNPs were analyzed by KASPar, technique of allele-specific amplification followed by fluorescence detection in real-time PCR (Cupen 2007).

Several quality controls were carried out in the genotyping analysis. Firstly, three controls of Centre d'Etude du Polymorphisme Humain (CEPH) samples (preference of mother, father and son) as positive controls were included in each plate for all the SNPs genotyped through MALDI-TOF. The genotype of all this samples was known as they are reported in the HapMap database (www.hapmap.org). Additionally, two negative controls were included on each plate, as well as 20% duplicate samples, whose allelic frequencies for European population were confirmed according to the NCBI database.

Statistical Analysis

Statistical calculations were made using PASW Statistics 18 (IBM SPSS Statistics) and R for Windows (González 2007). There were no adjustments for multiple comparisons due to most SNPs have already been previously reported (Saville 2003).

All dependent variables were log-transformed in order to enhance the normality of their distribution and the homogeneity of their variances. In the case of CD_{4+} cell counts and VL values were squared root transformed because this transformation is especially appropriate when the data are counts. For variables with several temporal measurements available, the median of these values was calculated for their use as covariate in the statistical tests in which only one observation by individual was analyzed.

Genotyping data were filtered using the genotype call rate (> 90% completeness), the Hardy-Weinberg equilibrium (HWE) test (p-value > 0.001) and a minor allele frequency (MAF) criterion (> 1%) in order to ensure adequate quality of the PG data. Genotype for each SNP was coded as 0 (homozygous for the most frequent allele) or 1 (heterozygous or rare allele homozygous) to avoid statistical problems related with an excessively low number of rare homozygous in some SNPs.

Linear regression was carried out to evaluate the association between individual SNP markers and LPV/r PK parameters (CL/F, V/F, C^{ss}_{max}, C^{ss}_{mean} and C^{ss}_{min}). Demographic variables (age, gender, race and body mass index [BMI]), RTV concentration, TFV coadministration, HCV coinfection and the median of biochemical data were included as covariates in the regression model.

Efficacy was evaluated through the longitudinal analysis of CD_{4+} cell count and VL. Age, gender, race, HCV coinfection and genotype for each SNP were included as fixed-time covariates. Time dependent covariates included in the regression model were BMI and CD_{4+} cell count (in the analysis of VL) or VL (in the analysis of CD_{4+}) to take into account the correlation between both efficacy measurements.

Plasma lipid levels and TB were analyzed longitudinally using a mixed generalized linear model, in which is possible to accommodate unbalanced repeated measurements made at irregular time intervals. Genetic association was tested for each SNP adjusting for some time-dependent covariates (BMI, CD_{4+} cell count and RTV concentration) and other time-fixed covariates (age, gender, race, HCV coinfection, TFV coadministration, CL/F, V/F and C^{ss}_{max}). Diarrhea was coded as 0 or 1 and used as dependent variable in a logistic regression to study genetic association adjusting for demographic variables, HCV coinfection, TFV coadministration, CD_{4+} cell count, RTV concentration and LPV/r plasma concentrations (C^{ss}_{max}, C^{ss}_{mean} and C^{ss}_{min}).

RESULTS

Study population and PK, efficacy and toxicity data

One hundred and nine HIV-infected patients treated with LPV/r were enrolled in the study for genotype-phenotype analysis as they fulfill all the inclusion criteria. The baseline demographic, clinical and biochemical data and PK parameters are summarized in Table 2. Most patients were European (97.3%) males (72%). A considerable percentage of patients (42%) had concentrations two-fold above 4 mg/L, that is the Cssmin recommended for PI-pretreated patients (Van der Leur 2006, Gutiérrez 2003, Masquelier 2002). The mean plasma lipid values were higher than the standard

recommended, 20% of the patients had median TB levels greater than the reference value of 1 mg/dL (the highest value was 5.3 mg/dL) and a considerable percentage of them presented diarrhea (25%). The 43% of the patients were coinfected with HCV. Besides this, most had a good clinical evolution because the mean CD_{4+} lymphocyte cell count was 400×10^6 cells/mL, and 67 patients (61.47%) had an undetectable plasma VL (<50 copies/mL). Regarding other ARV drugs analyzed, the 77% had TFV as a component of their cART.

Genetic polymorphisms

A total of 290 SNPs in the genes encoding for proteins involved in the ADME process of LPV/r, efficacy and toxicity related to the treatment were analyzed. The genotype data for all genetic polymorphisms assayed (including markers, gene, gene position, genotype frequencies, HWE, genotyping rate and MAF) are listed in Table 1. Most of the frequencies of known SNPs were in accordance with published data. However, 24 were monomorphic, 2 were not in HWE equilibrium and 11 showed a MAF lower than the used threshold of 0.01, so they were excluded from the final analysis. This resulted in a total of 253 SNPs available for statistical analysis.

Statistical analysis

The linear regression analysis of the effect of SNPs and demographic, clinical and biochemical covariates on PK parameters revealed a significant influence RTV concentration and suggestive effect of others covariates (TFV coadministration, BMI and gender) and genotype for 56 SNPs ($p < 0.05$) (data not shown). The Person correlation coefficient and probabilities for the RTV concentration and the PK parameters were: 0.7886-0.8243 ($p < 0.0001$) for the LPV/r concentrations, 0.6308 ($p <$

0.0001) for the CL/F and 0.1934 (p = 0.0439) for the V/F. Those associated SNPs showing a $p < 0.01$ in someone of the PK analysis are shown in Table 3, which were a total of nine. The most clear and significant association was of a SNP from dopamine receptor D3 (DRD3), rs1486012, which remained significant after adjusting for multiple testing (Bonferroni adjusted p-value = 0.0002, c orresponding to 253 t ested SNPs). Moreover the two SNPs in DRD3 (rs1486012 T>A and rs963468 G>A) were those that had a closer association with all LPV/r PK parameters ($p < 0.004$). Carriers of these genetics variations showed a lower elimination of LPV/r and consequently higher plasma concentrations.

Other PK parameters not shown in Table 3 were also evaluated. In relation with the C^{ss}_{mean}, the SNPs which showed a probability lower than 0.01 in the association analysis were those that showed it for the C^{ss}_{max}. However, in the case of V/F, no SNPs were identified with a significant probability.

The results of the longitudinal analysis of the associations between genotype and the efficacy parameters, CD_{4+} and VL, are shown in Table 4. Regarding to the effect of covariates, the VL was statistically significant in the analysis of CD_{4+}; however, none of them showed significant influence on the VL analysis. The longitudinal analysis for CD_{4+} revealed that one SNP in the stromal derived factor 1 (SDF-1), one in APOA, one in cadherine (CDH13) and another in ABCG2 genes showed significant influence on CD_{4+} cell counts (see Table 4). For the SNPs in SDF-1 and APOA genes, the association was negative, which means that the presence of the allelic variant led to lower CD_{4+} counts; on the other side, the rare allele of SNPs in CDH13 and in ABCG2 genes showed a protective effect, favoring higher CD_{4+} levels.

Regarding the toxicity analysis, due to the own characteristics of the data, it was carried out a longitudinal analysis for lipid plasma levels and TB and a logistic regression for diarrhea. The most frequently significant covariates were C^{ss}_{max}, CL/F, CD_{4+} cell counts, BMI, age and time. The longitudinal analysis revealed the influence of eight SNPs: five in CETP, one in ABCC2, one in LEP and another in the monocyte chemoattractant protein 1 (MCP-1) gene on the lipid and TB plasma levels (Table 5). In relation with the lipidic analysis, the suggestive associations were found with the SNPs in ABCC2, CETP and CYP3A4 genes, which led to higher cLDL and cHDL levels and cLDL/cHDL; while the SNPs LEP rs1137100 and MCP-1 rs4586 were associated with lower cHDL and TC levels, respectively. In relation to the TB analysis, the SNP rs11076174 in CETP gene was related with lower TB levels and none of the analyzed polymorphisms were associated with higher ones.

Regarding the logistic regression for the presence of diarrhea, the SNP rs2069835 in interleukin (IL)-6 gene showed the most significant influence on the development of this adverse effect ($b = 0.9718$, $p = 0.0048$).

DISCUSION

Medication dosage had been based on demographic and clinical parameters traditionally. However, during the LPV/r-based cART, a considerable IIV in plasma concentrations and response is observed (Moltó 2006, Van der Leur2006). Among the factors contributing to this variability, the best characterized have been those related with the anthropometric and clinical conditions of the patients, as demonstrated to be the BMI in this analysis; but the demographic factors age, gender and race showed a

lack of influence on PK behavior of LPV/r in agreement with previous results (data not shown) (Aspiroz 2011, Lubomirov 2010, Moltó 2008, Bouillon-Pichault 2009, Ribera 2004).

Recently, host genetic determinants have been appointed as an important cause of these unexplained remaining interpatient differences (Lubomirov 2010, Elens 2009, Owen 2008). The genetic factors explained more than 5% of the IIV in the CL/F in a large and well designed PK/PG population study carried out (Lubomirov 2010), which was lower than the 20% that was justified by the RTV trough plasma concentrations in a PK research by our group (Aspiroz 2011). However, with respect to the IIV on the VL during the asymptomatic period of the HIV infection, in a whole-genome association study some SNPs were found to explain nearly 15% of it (Fellay 2007).

In the univariate analysis carried out in the present study, a negative significant correlation was found between the CL/F of LPV/r and the RTV plasma concentrations, as can be expected because this is the basis of the boosting effect (Kumar 1998). However, this strong association could hide the real influence of genetic polymorphisms of genes responsible for metabolism and transport of LPV/r that could not be demonstrated. In contrast with other studies, where only LPV/r plasma concentrations reflecting the PK of this drug were available for the analysis (Elens 2009, Estrella 2008), in this study were incorporated individual PK parameters (CL/F and V/F). Moreover, these values and those of mean plasma concentrations along the dosage interval were estimated with a Bayesian approach in clinical PK software which included a population PK model previously developed with some of these patients (Aspiroz 2011).

Probably, the most important findings of this investigation were the strong associations demonstrated in a univariate analysis between the SNPs rs1486012 and rs963486 in the DRD3 gene and the PK parameters of LPV/r: CL/F, C^{ss}_{max}, C^{ss}_{mean} and C^{ss}_{min} (Table 3). For both SNPs, the presence of the rare allele A led to a decrease in drug elimination and thus, an increase in LPV/r plasma concentrations (Figure 1). In this sense, it is known that the dopamine modulates the hepatic blood flow (Peschl, 1978), and according to our results, maybe the rs1486012 and rs963486 rare alleles could affect the affinity of receptors for the dopamine, and a lower activity of the neurotransmitter could decrease the hepatic blood flow and hence the degree of LPV/r metabolism. Thus, expression studies of these SNPs are need to confirm these results due to the great prevalence of these SNPs among the European population (63.3 and 55.05% for rs1486012 and rs963486 respectively, in our study population).

Another unexpected finding suggested the influence of two SNPs in thiopurine methyltransferase (TPMT) gene, rs11422345 and rs1800460, on the PK of LPV/r. Each one of these genetic variants in heterozygosis conducted to a lower CL/F (0.050 and 0.045 L/kg/h for rare alleles of rs11422345 and rs1800460 *versus* 0.065 and 0.062 L/kg/h for the wild-type genotype of them, respectively), and hence higher LPV/r plasma concentrations. In relation to the effect observed, this transport protein could also be implicated in the LPV/r disposition.

In general, the influence found among the SNPs in CYPs genes on the PK parameters of LPV/r was of low relevance, which was in agreement with previous results (Lubomirov 2010, Elens 2009, Estrela 2008, Gupta 2008), in spite of LPV/r is mainly metabolized

by CYP3A enzymes (Kumar 1999). It was observed a slightly relationship between the rs28371764 (CYP3A5*1C allele) and the CL/F ($p = 0.02$) and the C^{ss}_{min} ($p = 0.027$). The effect of the presence of the rare allele, that led to greater metabolism and lower C^{ss}_{min}, had been already described for others drugs, for which dosage adjustment recommendations are based on the genotype of the rs28371764 (Mouly 2005, Haufroid 2006, Le Meur 2006). However, the contribution of the rs6945984 genetic variant to a lower value of CL/F reported by Lubomirov et al. 2010 could not be replicated by the present study despite of being represented in the 18.6% of the patients genotyped.

Although the CYP3A4_1461ins polymorphism showed a MAF < 1% and it did not fulfill the required criteria to be incorporated to the statistical analysis, an exception was considered with respect to the remaining SNPs with low MAF and it was explored due to the known importance of CYP3A4 in the LPV/r metabolism (Kumar 1999). The insertion of an A in this position characterizes the CYP3A4*20 allele, which represents the first CYP3A4 allele that has no functional activity as a result of a premature stop codon yielding a truncated protein (Westlind-Johnsson, 2006). As can be expected, the SNP showed a closer relationship with the C^{ss}_{min} ($p = 0.006$), which could be justified by the reduction in CYP3A4-mediated metabolism of LPV/r. In spite of the reported frequency of this rare variant allele is < 0.06% in white subjects (Westlind-Johnsson, 2006), one of the patients of this study presented the genetic variant in heterozygosis.

The influence of polymorphisms in the transport protein genes MDR1 and MRP-2 on LPV/r disposition and response still remains controversial (Winzer 2003, Fellay 2002, Ma 2007, Gupta 2008, Elens 2009, Zhang 2010). The present study did not observe any suggestive influence of SNPs in these genes on LPV/r plasma concentrations or PK

parameters in agreement with most of previous researches (Winzer 2003, Ma 2007, Gupta 2008, Elens 2009).

In relation to the OATP, several candidate SNPs and the tagSNPs of the SLCO1A2 gene were genotyped because some of them explained part of the LPV/r PK variability in recent publications (Lubomirov 2010, Hartkoorn 2010, Kohlraus 2010). For the rs4149056, the present study suggested the same association previously observed (Lubomirov 2010, Hartkoorn 2010, Kohlraus 2010). This is a non-synonymous SNP (521 T>C) associated with a decreased uptake of LPV/r by the hepatocytes, thus explains a reduction in CL/F and an increment in LPV/r plasma concentrations (Kameyama 2005, Niemí 2007, Lubomirov 2010, Kohlraus 2010). Beside this, no significative associations were found with the other SNPs analyzed according to some of the previously reported results (Lubomirov 2010, Hartkoorn 2010, Elens 2009, Kohlraus 2010).

Most of the genetic association studies in HIV-infected patients concerning genes related to lipid metabolism have focused on the study of the toxicity caused by cART or an ARV family, instead of a particular ARV agent. In these studies, the ARV agents were distributed into groups according to published reports of their lipid effects (Fontas 2004, Young 2005, Arnedo 2007). Thus, the genes candidates for the present analysis, were associated before with the toxicity of the PI-based therapy (Arnedo 2007, Chang 2009, Tarr 2005, Fauvel 2001, Foulkes 2006, Miserez 2001, Yang 2003). Now, some of these SNPs showed suggestive associations with the PK parameters of LPV/r, which could be indirectly related with the potential toxicity that can be developed during the treatment with this drug.

Cadherins, encoded by the CDH13 genes, are a family of membrane receptors that mediate calcium-dependent hemophilic cell adhesion and are responsible for formation of stable cell junctions and maintenance of normal tissue structure (Braga 1999). In the context of this research, it is of special interest their participation in lipoprotein-dependent signaling (Bochkov 2005, Boggon 2002) and the interaction with blood plasma lipoproteins (Braga 1999). In this sense, the CDH13 rs17216786 polymorphism which affects to the receptor of LDL, showed a negative correlation with the C^{ss}_{max} of LPV/r, caused by a higher CL/F, and a better recovery of CD_{4+} for patients carriers of the allelic variant. An alteration of the booster effect of RTV may explain this result however, the mean value of C^{ss}_{min} was unaffected, which could justify the favorable immune response of this group of patients. Although this finding could be interpreted as a protective effect of this polymorphism against LPV/r elevated concentrations, any relevant association was found in relation with this SNP in the toxicity analysis. Therefore, it cannot be ruled out that lipoprotein binding to cadherin may affect LPV/r disposition and response according to these results.

The microsomal TG transfer protein (MTTP) transfers lipids into APOB-containing lipoproteins for secretion from liver, intestine and heart. Thus, like APOE, the MTTP is potentially related to cardiovascular disease by regulation of the plasma concentration of cHDL (Musso 2009). In this sense, only a lightly tendency ($p = 0.02$) was observed between the SNPs rs2298747 T>A and the cLDL/cHDL in our population. The presence of the rare allele seemed to be associated with a higher coefficient, a bad prognostic factor of cardiovascular disease. Additionally, other polymorphisms in this gene had been reported to induce a loss of MTTP transfer activity due to an abnormal truncated

protein which could be responsible for several lipid disorders (Pons 2011). In the present study, the presence of the rare allele of the rs6532823, which has not been previously analyzed in this context, led to higher CL/F and consequently, lower C^{ss}_{min} (Table 3).

The resistin and the SREBP-1 genes are also related with the lipid metabolism, fat redistribution and insulin resistance (Carr 1998, Dowell 2000, Steppan 2001, Ranade 2008). The documented inhibition by the PIs of the SREBP-1, might have an impact on this kind of cART-related toxicity (Carr 1998, Dowell 2000). However, with respect to the influence of SNPs in these gene on these mechanisms, data are scarce and inconsistent (Miserez 2001, Yang 2003, Ranade 2008). The lack of influence of some SNPs on lipid levels was confirmed by the present study, which is in agreement with other results (Yang 2003). In contrast, two novel associations were found for the SNPs rs2297508 and rs3219177, in the SREBP-1 and resistin gene, respectively. It was observed a reduction on CL/F which conducted to higher LPV/r plasma concentrations than those of non-carriers of the genetic variants.

As can be seen, some of the genes which showed certain correlations with the PK of LPV/r in this study were related with the lipid metabolism, as MTTP, resistin and SREBP-1. Because of the lipophilic characteristics of LPV/r, these lipid transporters may participate in the transport of the drug which could affect to the ADME process.

The genes encoding for some of the proinflammatory cytokines such as the TNF-α, IL-1A and IL-6, had been widely explored looking for genetic polymorphisms responsible for interpatient differences in the metabolic syndrome caused by the cART, which

includes lipodystrophy and hyperlipidemia (Tarr 2005, Maher 2002, Nolan 2003, Saumoy 2008, Price 2004, Dominici 2002). However, currently available data concerning this issue are inconclusive (Gutiérrez 2008). The cART seems to alter the TNF-α homeostasis, thus insulin sensitivity and the differentiation of adipocytes, as well as transducing apoptotic stimuli, could be modified in this clinical situation (Sethi 1999, Qian 2001, Ledru 2000). Among the SNPs analyzed in the TNF-α gene, the rs3093662 was associated with a reduction in the CL/F. However, because this variation did not produce any significant effect on LPV/r plasma concentrations, another mechanism of compensation could be happening responsible for this effect.

Regarding the efficacy analysis, the CDH13 rs17216786 and the ABCG2 rs2231137 were associated to higher CD_{4+}. In relation with the latter finding affecting the transport protein, there is one report where the MDR1 3435TT genotype was also associated with greater mean CD_{4+} rise, where most of the patients were long-term viral suppressed like our population, but the follow-up period was 24 weeks, which is much shorter than this of the present study (Fellay 2002). Besides this, in agreement with other studies it was not observed any association between the MDR1 3435TT genotype and the increase of CD_{4+} or the decline of the VL in spite of the adequate representation of the genotypic variant (73% of the population presented the rare allele) (Nasi 2003, Winzer 2005).

On the other hand, the allelic variant of the SDF-1 rs266087 led to lower CD_{4+} cell counts (Table 4). This clinical situation characterized by lower CD_{4+} cell counts and a decrease in plasma SDF-1 levels has been observed during the most advanced stages of the HIV-1 infection, whereas high plasma SDF-1 levels has been associated with long-term nonprogression (Soriano, 2002). In spite of this SNP is located in an intron, and

thus it is unlikely that it could affect the expression of the gene by itself, the rs266087 could be in linkage disequilibrium with another SNP which could regulate the expression of SDF-1 according to the results observed in this analysis. In agreement with other study, the genetic variant of rs1801157 showed a weak association ($p = 0.05$) with lower CD_{4+} cell counts, due to the lower plasma concentration of SDF-1 in individuals homozygous for the mutant allele that was demonstrated (Coll 2005).

The control of the viral replication has shown also to be partially modulated by genetic factors (Fellay 2007) and the identification of patients at greatest risk of virological failure could allow the selection of the most appropriate cART to achieve the maximal efficacy and durability. In this study it was observed that the rare allelic variants of SDF-1 rs266092 and IL-6 rs2069837 were associated with an unfavorable virological response of HIV-patients receiving LPV/r based cART. Because of the SDF-1 rs266092 is located in the promoter of the gene, the polymorphism could affect the expression of SDF-1; however, as the IL-6 rs2069837 is within an intron, if an alteration in the transcription happened, it would be in linkage disequilibrium with other SNP.

In the present study, some SNPs of the genes ABCC2 and CETP showed an association with an increment on lipid levels (TC, cLDL, cHDL) and/or cLDL/cHDL coefficient in patients taking LPV/r as part of their cART. In relation to the CEPT gene, these results confirmed other reported in the literature, with the exception of the decrease on TG for rs708272 observed by Arnedo et al. 2007. For the ABCC2 rs3740066, its influence on lipid elevation was not surprising as this is not the first evidence that a SNP in a transporter protein gene showed a similar effect. In this sense, the ABCA1 rs4149313

polymorphism which caused elevation in TC, cLDL and cLDL/cHDL ($0.01 < p < 0.04$), showed to lead to higher TG levels in other study (Arnedo 2007).

On the other hand, the LEP rs1137100 and the MCP-1 rs4586 and rs13900, contributed to lower lipid levels, effect that had not been reported to date as far as we know. The implication of the LEP gene has been studied in obesity context and it appears to provide moderate protection to infants from an excess of weight gain (Miralles 2006). In relation with the MCP-1 rs4586C and rs13900 T rare alleles, they could reduce the activity of the protein causing a protective effect against plasma lipid elevations (TG, TC, cLDL). In contrast with these effects, the MCP-1–2518G polymorphism, which is in the distal regulatory region of the MCP-1 gene, was associated with a higher expression of the protein and five-fold increase of the risk for atherosclerosis in HIV-infected patients in other researches (Rovin 1999, Alonso-Villaverde 2004). Therefore, the effect of these SNPs in LEP and MCP-1 genes on the activity of the protein and its role on lipid metabolism should be studied in future research to confirm the apparent protective effect against hyperlipidemia observed by these results.

Regarding the SNPs in the APO genes, only the APOE rs7412 showed a suggestive influence on the increment of cHDL and the reduction of cLDL/cHDL, while other authors found higher TG levels due to a RTV-based cART (Arnedo 2007, Tarr 2005). In relation to the APOA5 rs662799, APOC3 rs5128 and the APOE rs429358, in spite of their relevant presence in the sample population, the results showed a lack of effect on plasma lipid elevations in contrast with other studies of the literature (Tarr 2005, Arnedo 2007, Chang 2009, Fauvel 2001, Foulkes 2006).

Concerning the TNF-α gene, it was confirmed the lack of influence of the rs361525 (-238 G>A) on lipid levels (Arnedo 2007). Other candidate SNPs located in SREBP-1 and Transcription factor 7-like 2 (TCF7L2) genes, neither contributed significantly to plasma lipid levels in the present dataset.

It has been hypothesized that the PIs induce peripheral lipodystrophy by binding and inhibiting proteins that are involved in lipid metabolism, the cytoplasmic retinoid-acid binding protein type 1 (CRABP-1) and the low density lipoprotein-receptor-related protein (LRP). PIs have high affinity for the catalytic site of HIV protease, which has approximately 60% homology to regions within these two proteins (Carr 1998). Thus, when the PIs bind to CRABP-1, the result is an impaired CRABP-1-mediated cis-9-retinoic acid stimulation of RXR:PPAR-γ, resulting in reduced differentiation and increased apoptosis of peripheral adipocytes (Carr 1998). Both of these circumstances cause hyperlipidemia by reducing triglyceride storage and lipid release into the circulation (Carr2000). When some SNPs in the CRABP-1 gene were analyzed, it was observed a suggestive association ($0.02 < p < 0.04$) between the rare allele carriers (rs3784333 G>A, rs4886568 T>C) and a reduction in TG levels. In relation to the cHDL levels, while the rs2139440 C>A was associated with a decrease, for the rs4886568 T>C was an increment. According to these results, these SNPs may lead to a relevant alteration in CRABP-1 binding site, that could avoid the PI binding and thus, it would justify the observed protective effect against lipid increments or the apparent less toxicity by LPV/r.

Among the PK data reflecting exposure to LPV/r, only the C^{ss}_{max} demonstrated to be a significant covariate in the longitudinal analysis of TC, cLDL and cLDL/cHDL.

Although some authors found a correlation between LPV/r trough plasma concentrations and elevated lipid levels (Gutiérrez 2003, González de Requena 2003), the lack of significance of this relationship is concordant with other studies which observed no short-time relation between PIs (LPV and saquinavir) trough concentrations and blood lipid levels in heavily pretreated patients (Clevenberg 2003).

In spite of hepatobiliary disorders are not common during the treatment with this LPV/r, the influence of the genetic factors was explored in relation to TB levels due to the high values observed in the studied population (Table 2). Two SNPs in the CETP gene, rs11076174 and rs9939224, were associated to TB levels, showing a protective effect for TB elevations of the rare allelic variants. No SNPs seemed responsible for an increment in the value of this biochemical parameter.

The gastrointestinal side effects are frequently caused by the LPV/r-based cART, and the diarrhea is one of them that often compromise the quality of life of the patients. The identification of a genetic predisposition to suffer it could be a criterion to take into account in the treatment with LPV/r. In this investigation, it was not considered the presence of this adverse effect associated with the administration of LPV/r in soft capsules, because one of the excipients of this formulation seems to be responsible for it. Regarding the IL-6 gene, the presence of the rare allele rs2069835 C in heterozygosis, which was present in more than 20% of the studied population (no homozygous genotype CC was found), showed an influence with the presence of diarrhea ($p < 0.005$). This association was also suggested for the IL-1β rs1071676 and the rs1143634 rare alleles ($p < 0.02$) as well as for other genetic variations in transporter proteins genes in a lower degree ($p < 0.05$). It is known that when diarrhea appears in

irritable bowel syndrome, the clinical situation is characterized by an augmented cellular immune response with enhanced production of proinflammatory cytokines, indeed a higher baseline IL-6, IL-1β and TNF- α levels were observed in these patients in comparison with healthy controls (Liebregts 2007). If the rare alleles of those SNP in IL-6 and IL-1β genes that showed the association in this study conducted to higher cytokine production, it could contribute to the development of diarrhea in a greater degree according to the results observed. Finally, it is noteworthy that the status of diarrhea was established by self-report and therefore some people classified as having this side effect could have hidden or underestimated exposure to substances causing also diarrhea. Thus, these encouraging findings need to be confirmed due to the potential implication in clinical decisions.

Several reasons could explain the discrepant results obtained in this study. The 8.27% of the selected SNPs were monomorphic and 3.79% of them showed a low MAF in Europeans, which makes difficult to evaluate their influence on the PK of LPV/r or the response. Those monomorphic SNPs could not be ruled out in the SNP selection as this information was not specified on the available database for Europeans. Besides, as the 97% of the population of this study was European, differences attributable to other races could not be established. As in other studies, a higher proportion of men had been included in the analysis which could be another of the limitations (Arnedo 2007, buscar más). Therefore, future studies need to include a greater proportion of patients of non-European origin, similar proportion between both genders and a larger sample size.

Other of the limitations which affected to the toxicity analysis was the absence of lipids measurements previously to the beginning of the LPV/r-based therapy. However, at the

onset of the study the patients could have already developed the metabolic syndrome with abnormal plasma lipid levels due to the HIV replication, chronic hepatitis coinfections and the cART (Piafsky 1980, Lemaire 1986). Besides this, most of the patients included in the study were heavily pretreated, thus these lipid data could not be relevant for the study because the increase in lipid levels occur in the first four weeks since the beginning of the treatment with PIs (Rhee 2010). Indeed, the mean TG and TC values of our population were 212.94 ± 128.82 and 187.12 ± 45.22 mg/dL respectively, higher than the upper limits recommended (200 and 150 mg/dL, respectively). Moreover, no consistently information about lipid-lowering therapy was available because it is often controlled by the primary care physician instead of by the specialist at hospital. Although this may be another limitation of the study, the use of these drugs was allowed in some similar designed studies (Gutiérrez 2003).

Because this is a replication study which were analyzed in single analysis the totally of the SNPs in candidate genes according to the literature, no adjustments for multiple testing were performed (Saville 1990, Roback 2003). The number of SNPs analyzed in other studies was often low; besides the mechanisms which determine the drug disposition and response to the treatment with LPV/r are often complex and they are not well defined. Thus, these facts contribute to the known difficulty to replicate and confirm the previously reported genetic associations, which could justify some of the differences found in this research. In this sense, some results must be taken with caution and the probabilities could be considered suggestive rather than clearly significant because other additional SNPs related to the efficacy and toxicity of other ARVs, different from LPV/r, were also analyzed.

In conclusion, this work performed a comprehensive PG analysis of genes encoding the proteins involved in the ADME process of LPV/r as well as other which participate in the response and toxicity of the treatment with this drug. The most significative associations were found between the SNPs in DRD3 gene and the PK parameters of LPV/r; additionally, some suggestive relationships has been established between genetic factors and the PK, efficacy and toxicity parameters that characterize the studied drug. As some of these results have not been previously reported, gene expression and functional studies evaluating the effect of these SNPs should be carried out. Moreover, replication analysis should confirm the results obtained in this study previously to its application in clinical practice to achieve a more effective and safer LPV/r-based cART.

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Table 1. Genotype data of analyzed SNPs

GENE	SNP	GENOTYPE				QUALITY CONTROL		
		Total (N)	Wild-type (%)	Heterozygous (%)	Homozygous (%)	HWE (prob)	Genotype Rate (%)	MAF
ABCA1	rs4149313 A>G	109	70.64	25.69	3.67	0.48	100	0.165
ABCB1	rs2032582 G>T	107	53.27	38.32	8.41	0.67	100	0.276
ABCB1	rs1045642 C>T	109	33.03	44.95	22.02	0.35	100	0.445
ABCB1	rs1128503 C>T	109	46.79	42.2	11.01	0.74	100	0.321
ABCB1	rs2229109 G>A	109	98.17	1.83	0	0.92	100	0.009
ABCB1	rs2235046 G>A	108	45.37	43.52	11.11	0.89	99.08	0.329
ABCB1	rs3789243 T>C	109	39.45	44.95	15.6	0.63	100	0.381
ABCB1	rs9282564 A>G	108	90.74	8.33	0.93	0.15	99.08	0.051
ABCC1	rs246221 T>C	95	53.68	36.84	9.47	0.41	87.16	0.279
ABCC1	rs35587 T>C	109	44.04	45.87	10.09	0.70	100	0.330
ABCC1	rs45560437 G>A	109	84.4	15.6	0	0.38	100	0.078
ABCC2	ABCC2_259 G>T	108	96.3	3.7	0	0.84	99.08	0.019
ABCC2	rs17222723 T>A	108	88.89	11.11	0	0.54	99.08	0.056
ABCC2	rs2273697 G>A	107	63.55	30.84	5.61	0.46	98.17	0.210
ABCC2	rs3740066 G>A	98	38.78	44.9	16.33	0.59	89.91	0.388
ABCC2	rs7080681 G>A	109	100	0	0		100	0.000
ABCC2	rs717620 G>A	108	62.04	30.56	7.41	0.18	99.08	0.227
ABCC2	rs8187707 C>T	109	88.07	11.93	0	0.51	100	0.060
ABCC4	rs11568658 G>T	108	96.3	3.7	0	0.84	99.08	0.019
ABCC4	rs11568695 G>A	109	98.17	1.83	0	0.92	100	0.009
ABCC4	rs12875235 T>G	107	73.83	25.23	0.93	0.43	98.17	0.136
ABCC4	rs1557070 C>T	109	98.17	1.83	0	0.92	100	0.009
ABCC4	rs1751034 T>C	109	60.55	37.61	1.83	0.12	100	0.206
ABCC4	rs2274405 C>T	109	34.86	50.46	14.68	0.59	100	0.399
ABCC4	rs2274406 C>T	108	33.33	50	16.67	0.77	99.08	0.417
ABCC4	rs2274407 C>A	109	85.32	14.68	0	0.41	100	0.073
ABCC4	rs3742106 A>C	109	34.86	51.38	13.76	0.43	100	0.394
ABCC4	rs45616431 T>C	108	98.15	1.85	0	0.92	99.08	0.009
ABCC4	rs899494 G>A	107	67.29	24.3	8.41	0.01	98.17	0.206
ABCG2	M376CT	108	100	0	0		99.08	0.000
ABCG2	rs2231137 G>A	108	87.96	12.04	0	0.51	99.08	0.060
ABCG2	rs2231142 C>A	108	86.11	13.89	0	0.44	99.08	0.069
CYP1A2	rs762551 A>C	105	43.81	44.76	11.43	1.00	96.33	0.338
CYP1A2	CYP1A _3534GA	108	100	0	0		99.08	0.000
CYP1A2	rs72547513 C>A	109	100	0	0		100	0.000
CYP1A2	CYP1A2_634AT	109	100	0	0		100	0.000

CYP1A2	rs2069526 T>G	108	98.15	1.85	0	0.92	99.08	0.009
CYP2B6	rs3211371 C>T	109	78.9	21.1	0	0.22	100	0.106
CYP2B6	rs3745274 G>T	109	59.63	35.78	4.59	0.78	100	0.225
CYP2B6	rs8192709 C>T	109	88.99	11.01	0	0.54	100	0.055
CYP2C19	rs12248560 C>T	109	59.63	35.78	4.59	0.78	100	0.225
CYP2C19	rs41291556 T>C	109	99.08	0.92	0	0.96	100	0.005
CYP2C19	rs4244285 G>A	109	81.65	16.51	1.83	0.35	100	0.101
CYP2C19	rs4986893 G>A	109	100	0	0		100	0.000
CYP2C8	rs10509681 T>C	109	68.81	29.36	1.83	0.50	100	0.165
CYP2C8	rs11572080 G>A	109	68.81	29.36	1.83	0.50	100	0.165
CYP2C8	rs11572103 A>T	104	94.23	5.77	0	0.76	95.41	0.029
CYP2C9	rs1057909	109	100	0	0		100	0.000
CYP2C9	rs1057910 A>C	106	100	0	0		97.25	0.000
CYP2C9	rs1057911 A>T	109	84.4	15.6	0	0.38	100	0.078
CYP2C9	rs2256871 T>C	109	99.08	0.92	0	0.96	100	0.005
CYP2C9	rs28371685 C>T	109	100	0	0		100	0.000
CYP2C9	rs9332130 A>G	109	100	0	0		100	0.000
CYP2C9	rs9332239 C>T	109	100	0	0		100	0.000
CYP3A	rs6945984 T>C	102	81.37	17.65	0.98	0.98	93.58	0.098
CYP3A4	1461_1462insA	109	99.08	0.92	0	0.96	100	0.005
CYP3A4	rs4987161	109	100	0	0		100	0.000
CYP3A4	rs12721629	109	100	0	0		100	0.000
CYP3A4	P416L	109	100	0	0		100	0.000
CYP3A4	R130Q	109	100	0	0		100	0.000
CYP3A4	T185SmodC	109	100	0	0		100	0.000
CYP3A4	T185SmodT	109	100	0	0		100	0.000
CYP3A4	T363M	109	100	0	0		100	0.000
CYP3A4	rs2740574 A>G	109	88.99	9.17	1.83	0.01	100	0.064
CYP3A4	rs28371759 T>G	109	100	0	0		100	0.000
CYP3A5	rs10264272 C>T	109	100	0	0		100	0.000
CYP3A5	rs28365085 T>C	109	100	0	0		100	0.000
CYP3A5	rs28371764 C>T	109	90.83	9.17	0	0.62	100	0.046
CYP3A5	rs41279854 A>G	109	100	0	0		100	0.000
CYP3A5	rs4646453 G>T	109	96.33	3.67	0	0.85	100	0.018
CYP3A7	rs2257401 G>C	108	88.89	11.11	0	0.54	99.08	0.056
DRD2	141C_del	108	79.63	17.59	2.78	0.14	99.08	0.120
DRD2	Ser311Cys C>G	109	97.25	2.75	0	0.88	100	0.014
DRD3	rs1486012 T>A	109	36.7	44.95	18.35	0.47	100	0.408
DRD3	rs2134655 G>A	109	48.62	44.04	7.34	0.52	100	0.294
DRD3	rs963468 G>A	109	44.95	40.37	14.68	0.25	100	0.349
DYPYP	DPYP_2A G>A	109	100	0	0		100	0.000

NR1I2_PXR	rs1523130 G>A	109	36.7	42.2	21.1	0.16	100	0.422
NR1I2_PXR	rs2472677 T>C	107	32.71	49.53	17.76	0.89	98.17	0.425
NR1I2_PXR	rs763645 C>T	109	23.85	58.72	17.43	0.06	100	0.468
ORM1	ORM1_113 A>G	105	100	0	0		96.33	0.000
ORM1	rs10982151 T>G	108	66.67	31.48	1.85	0.37	99.08	0.176
ORM1	rs10982158 A>T	105	69.52	30.48	0	0.07	96.33	0.152
SLC22A6	rs4149170 G>A	105	80	18.1	1.9	0.46	96.33	0.110
SLCO1A2	rs10841795 A>G	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs10841798 T>G	109	88.07	11.93	0	0.51	100	0.060
SLCO1A2	rs10841803 G>A	109	69.72	28.44	1.83	0.57	100	0.161
SLCO1A2	rs11045918 C>A	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs11045923 C>G	108	35.19	52.78	12.04	0.23	99.08	0.384
SLCO1A2	rs11568563 A>C	109	88.99	11.01	0	0.54	100	0.055
SLCO1A2	rs11837182 C>T	109	81.65	17.43	0.92	0.99	100	0.096
SLCO1A2	rs16923597 A>G	109	66.97	28.44	4.59	0.47	100	0.188
SLCO1A2	rs4149006 C>A	108	66.67	30.56	2.78	0.73	99.08	0.181
SLCO1A2	rs4149008 C>T	109	67.89	27.52	4.59	0.40	100	0.183
SLCO1A2	rs4337089 C>T	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs5484 C>T	109	72.48	23.85	3.67	0.33	100	0.156
SLCO1A2	rs6487216 T>C	109	39.45	48.62	11.93	0.59	100	0.362
SLCO1A2	rs722994 G>A	107	74.77	20.56	4.67	0.05	98.17	0.150
SLCO1A2	rs7967770 A>C	107	78.5	20.56	0.93	0.74	98.17	0.112
SLCO1B1	rs11045819 C>A	109	71.56	24.77	3.67	0.40	100	0.161
SLCO1B1	rs17328763 T>C	108	60.19	33.33	6.48	0.51	99.08	0.231
SLCO1B1	rs2306283 T>C	109	29.36	49.54	21.1	0.98	100	0.459
SLCO1B1	rs4149015 G>A	109	83.49	14.68	1.83	0.21	100	0.092
SLCO1B1	rs4149032 C>T	109	35.78	45.87	18.35	0.57	100	0.413
SLCO1B1	rs4149056 T>C	109	73.39	22.94	3.67	0.26	100	0.151
SLCO1B3	rs4149117 G>T	109	79.82	16.51	3.67	0.03	100	0.119
SLCO2B1	rs1077858 A>G	109	42.2	44.95	12.84	0.87	100	0.353
TPMT	rs1142345 A>G	109	95.41	4.59	0	0.81	100	0.023
TPMT	rs1800460 G>A	108	96.3	3.7	0	0.84	99.08	0.019
5HT2A	5HT2A _102TC	109	27.52	54.13	18.35	0.83	100	0.454
5HT2A	HIS452TYR	109	79.82	19.27	0.92	0.00	100	0.106
5HT2C	CYS23SER	109	77.06	11.01	11.93	0.48	100	0.174
ADRB2	rs1042713 G>A	108	35,19	47,22	17,59	0,79	99,08	0,412
ADRB2	rs1042714 C>G	103	38,83	50,49	10,68	0,33	94,5	0,359
ADRB2	rs1042717 G>A	107	60,75	34,58	4,67	0,93	98,17	0,220
ADRB2	rs1042718 C>A	109	65,14	30,28	4,59	0,65	100	0,197
ADRB2	rs1042719 G>C	108	47,22	41,67	11,11	0,66	99,08	0,319
ADRB2	rs1800888 C>T	107	95,33	4,67	0	0,80	98,17	0,023

APOA	rs3135506 G>C	109	82,57	17,43	0	0,32	100	0,087
APOA	rs619054 C>T	109	54,13	40,37	5,5	0,55	100	0,257
APOA	rs651821 T>C	109	88,99	11,01	0	0,54	100	0,055
APOA	rs662799 A>G	108	88,89	11,11	0	0,54	99,08	0,056
APOA1	rs12718464 G>A	109	78,9	21,1	0	0,22	100	0,106
APOA1	rs2070665 C>T	109	87,16	11,93	0,92	0,47	100	0,069
APOA1	rs5072 C>T	109	87,16	11,93	0,92	0,47	100	0,069
APOC3	rs2070669 C>G	109	39,45	49,54	11,01	0,42	100	0,358
APOC3	rs4520 C>T	108	59,26	34,26	6,48	0,60	99,08	0,236
APOC3	rs5128 G>C	92	86,96	10,87	2,17	0,03	84,4	0,076
APOE	rs429358 T>C	109	86,24	12,84	0,92	0,56	100	0,073
APOE	rs7412 C>T	109	92,66	7,34	0	0,69	100	0,037
ASTN2	rs4838255 A>T	109	67,89	30,28	1,83	0,44	100	0,170
CDH13	rs17216786 C>A	109	81,65	17,43	0,92	0,99	100	0,096
CETP	rs11076174 T>C	109	80,73	18,35	0,92	0,91	100	0,101
CETP	rs11076175 A>G	109	61,47	35,78	2,75	0,34	100	0,206
CETP	rs11508026 C>T	105	39,05	42,86	18,1	0,29	96,33	0,395
CETP	rs12708974 C>T	109	78,9	19,27	1,83	0,59	100	0,115
CETP	rs12720898 C>T	109	84,4	15,6	0	0,38	100	0,078
CETP	rs1532624 G>T	107	36,45	47,66	15,89	0,96	98,17	0,397
CETP	rs1800777 G>A	109	94,5	5,5	0	0,77	100	0,028
CETP	rs1801706 G>A	109	67,89	28,44	3,67	0,74	100	0,179
CETP	rs1800775 C>A	109	27.52	47.71	24.77	0.64	100	0.486
CETP	rs1864163 G>A	109	43,12	46,79	10,09	0,60	100	0,335
CETP	rs2033254 T>C	109	41,28	47,71	11,01	0,60	100	0,349
CETP	rs289714 T>C	105	57,14	35,24	7,62	0,50	96,33	0,252
CETP	rs289715 A>T	109	75,23	22,94	1,83	0,95	100	0,133
CETP	rs289717 C>T	109	48,62	44,04	7,34	0,52	100	0,294
CETP	rs4784744 G>A	109	47,71	44,95	7,34	0,44	100	0,298
CETP	rs4784745 A>G	109	45,87	46,79	7,34	0,30	100	0,307
CETP	rs5880 G>C	108	91,67	8,33	0	0,65	99,08	0,042
CETP	rs5882 A>G	109	40,37	45,87	13,76	0,89	100	0,367
CETP	rs5883 C>T	109	91,74	8,26	0	0,65	100	0,041
CETP	rs708272 C>T	109	34,86	47,71	17,43	0,87	100	0,413
CETP	rs7203984 A>C	109	58,72	34,86	6,42	0,67	100	0,239
CETP	rs7205804 G>A	109	35,78	47,71	16,51	0,92	100	0,404
CETP	rs7499892 C>T	109	60,55	36,7	2,75	0,29	100	0,211
CETP	rs9926440 G>C	108	50	39,81	10,19	0,58	99,08	0,301
CETP	rs9929488 G>C	109	53,21	38,53	8,26	0,72	100	0,275
CETP	rs9939224 G>T	108	55,56	38,89	5,56	0,70	99,08	0,250
CLMN	rs1187614 C>A	109	66,06	31,19	2,75	0,67	100	0,183

CRABP-1	rs1127472 G>C	109	35,78	45,87	18,35	0,57	100	0,413
CRABP-1	rs12593362 G>T	109	35,78	45,87	18,35	0,57	100	0,413
CRABP-1	rs2139440 C>A	109	59,63	36,7	3,67	0,47	100	0,220
CRABP-1	rs3784333 G>A	109	88,99	11,01	0	0,54	100	0,055
CRABP-1	rs4886568 T>C	108	87,04	12,04	0,93	0,48	99,08	0,069
CRABP-1	rs8026332 G>C	109	86,24	13,76	0	0,44	100	0,069
FHOD3	rs17651157 T>C	109	87,16	12,84	0	0,47	100	0,064
IL1A	rs1304037 A>G	109	51,38	38,53	10,09	0,46	100	0,294
IL1A	rs1609682 A>C	109	48,62	41,28	10,09	0,75	100	0,307
IL1A	rs17561 G>T	109	53,21	36,7	10,09	0,30	100	0,284
IL1A	rs1800587 C>T	109	51,38	38,53	10,09	0,46	100	0,294
IL1A	rs20540 C>T	109	98,17	1,83	0	0,92	100	0,009
IL1A	rs2856836 T>C	109	53,21	36,7	10,09	0,30	100	0,284
IL1A	rs2856837 C>T	109	53,21	36,7	10,09	0,30	100	0,284
IL1A	rs3783525 T>A	109	48,62	41,28	10,09	0,75	100	0,307
IL1A	rs3783526 G>A	109	48,62	41,28	10,09	0,75	100	0,307
IL1A	rs3783550 A>C	109	48,62	41,28	10,09	0,75	100	0,307
IL1beta	rs1071676 G>C	109	70,64	26,61	2,75	0,89	100	0,161
IL1beta	rs1143630 C>A	109	83,49	15,6	0,92	0,84	100	0,087
IL1beta	rs1143634 C>T	108	70,37	26,85	2,78	0,91	99,08	0,162
IL1beta	rs1143643 G>A	108	40,74	48,15	11,11	0,56	99,08	0,352
IL1beta	rs3136558 T>C	107	66,36	29,91	3,74	0,87	98,17	0,187
IL6	rs1474347 T>G	104	36,54	48,08	15,38	0,95	95,41	0,394
IL6	rs1548216 G>C	109	92,66	7,34	0	0,69	100	0,037
IL6	rs1554606 G>T	109	32,11	51,38	16,51	0,58	100	0,422
IL6	rs1800795 G>C	109	36,7	48,62	14,68	0,82	100	0,390
IL6	rs1800797 G>A	109	38,53	46,79	14,68	0,94	100	0,381
IL6	rs2066992 G>T	109	85,32	13,76	0,92	0,65	100	0,078
IL6	rs2069832 G>A	109	37,61	47,71	14,68	0,94	100	0,385
IL6	rs2069835 T>C	109	79,82	20,18	0	0,24	100	0,101
IL6	rs2069837 A>G	109	83,49	16,51	0	0,35	100	0,083
IL6	rs2069840 C>G	109	51,38	41,28	7,34	0,80	100	0,280
IL6	rs2069843 G>A	109	92,66	7,34	0	0,69	100	0,037
IL6	rs2069849 C>T	109	92,66	7,34	0	0,69	100	0,037
KIRREL3	rs620875 T>G	108	68,52	29,63	1,85	0,49	99,08	0,167
LEP	rs10244329 T>A	109	30,28	50,46	19,27	0,82	100	0,445
LEP	rs10264361 T>A	106	86,79	12,26	0,94	0,49	97,25	0,071
LEP	rs10954173 G>A	109	43,12	42,2	14,68	0,39	100	0,358
LEP	rs1137100 A>G	109	62,39	33,03	4,59	0,93	100	0,211
LEP	rs1137101 A>G	109	33,03	55,05	11,93	0,11	100	0,394
LEP	rs11760956 G>A	109	43,12	42,2	14,68	0,39	100	0,358

LEP	rs11763517 C>T	109	25,69	53,21	21,1	0,49	100	0,477
LEP	rs12706832 G>A	109	28,44	46,79	24,77	0,51	100	0,482
LEP	rs1805134 T>C	109	64,22	32,11	3,67	0,88	100	0,197
LEP	rs2060713 C>T	109	87,16	11,93	0,92	0,47	100	0,069
LEP	rs2071045 T>C	108	54,63	39,81	5,56	0,61	99,08	0,255
LEP	rs2122627 C>T	109	86,24	12,84	0,92	0,56	100	0,073
LEP	rs2167270 G>A	104	42,31	43,27	14,42	0,53	95,41	0,361
LEP	rs2278815 A>G	109	28,44	45,87	25,69	0,39	100	0,486
LEP	rs28954099 C>T	109	94,5	5,5	0	0,77	100	0,028
LEP	rs28954101 A>G	109	88,07	11,93	0	0,51	100	0,060
LEP	rs3828942 G>A	109	39,45	48,62	11,93	0,59	100	0,362
LEP	rs4236625 A>T	109	85,32	13,76	0,92	0,65	100	0,078
LEP	rs4731426 C>G	108	28,7	45,37	25,93	0,34	99,08	0,486
LEP	rs7788818 G>A	109	87,16	11,93	0,92	0,47	100	0,069
LEP	rs7795794 G>A	109	87,16	11,93	0,92	0,47	100	0,069
LEP	rs8179183 G>C	109	64,22	33,03	2,75	0,52	100	0,193
LIPD	rs17410577 G>C	109	62,39	33,94	3,67	0,71	100	0,206
LIPD	rs10099160 T>G	105	64,76	29,52	5,71	0,34	96,33	0,205
LIPD	rs11570892 A>G	109	72,48	24,77	2,75	0,71	100	0,151
LIPD	rs1534649 G>T	109	33,94	43,12	22,94	0,18	100	0,445
LIPD	rs248 G>A	109	86,24	13,76	0	0,44	100	0,069
LIPD	rs249 T>C	109	88,99	11,01	0	0,54	100	0,055
LIPD	rs258 G>C	109	31,19	45,87	22,94	0,43	100	0,459
LIPD	rs281 T>A	109	53,21	36,7	10,09	0,30	100	0,284
LIPD	rs283 G>A	108	60,19	33,33	6,48	0,51	99,08	0,231
LIPD	rs285 C>T	109	32,11	43,12	24,77	0,17	100	0,463
LIPD	rs3779788 C>T	108	75,93	22,22	1,85	0,87	99,08	0,130
MCP-1	rs13900 C>T	109	62,39	31,19	6,42	0,34	100	0,220
MCP-1	rs4586 T>C	109	44,04	43,12	12,84	0,64	100	0,344
MTTP	rs1057613 A>G	109	33,94	44,04	22,02	0,27	100	0,440
MTTP	rs11937107 C>T	109	52,29	36,7	11,01	0,23	100	0,294
MTTP	rs17029163 A>G	109	77,98	20,18	1,83	0,68	100	0,119
MTTP	rs17029215 A>C	109	88,99	11,01	0	0,54	100	0,055
MTTP	rs2035816 A>G	109	69,72	27,52	2,75	0,98	100	0,165
MTTP	rs2255119 T>C	109	81,65	17,43	0,92	0,99	100	0,096
MTTP	rs2298747 T>A	109	69,72	28,44	1,83	0,57	100	0,161
MTTP	rs2306985 C>G	109	29,36	48,62	22,02	0,82	100	0,463
MTTP	rs3816873 T>C	108	52,78	36,11	11,11	0,19	99,08	0,292
MTTP	rs3828542 T>G	109	64,22	33,94	1,83	0,24	100	0,188
MTTP	rs6532823 C>A	109	67,89	29,36	2,75	0,84	100	0,174
MTTP	rs7698798 A>C	109	29,36	48,62	22,02	0,82	100	0,463

MTTP	rs982424 T>C	108	87,96	12,04	0	0,51	99,08	0,060
PPARγ	rs1801282 C>G	109	89,91	8,26	1,83	0,01	100	0,060
PPARγ	rs2920502 C>G	103	46,6	45,63	7,77	0,45	94,5	0,306
PPARγ	rs3856806 C>T	109	88,07	9,17	2,75	0,00	100	0,073
PPARγ	rs7638903 G>A	109	88,99	9,17	1,83	0,01	100	0,064
PPARγ	rs7649970 C>T	109	88,99	9,17	1,83	0,01	100	0,064
PPARγ	rs9870196 A>G	109	96,33	2,75	0,92	0,00	100	0,023
PRKAR2B	rs13224682 A>G	109	86,24	13,76	0	0,44	100	0,069
RESISTIN	rs1862513 G>C	108	56,48	36,11	7,41	0,61	99,08	0,255
RESISTIN	rs3219175 G>A	108	98,15	1,85	0	0,92	99,08	0,009
RESISTIN	rs3219177 C>T	108	70,37	25,93	3,7	0,49	99,08	0,167
RESISTIN	rs3219178 C>G	105	36,19	45,71	18,1	0,57	96,33	0,410
RESISTIN	rs3760678 A>G	106	83,02	15,09	1,89	0,23	97,25	0,094
RNF144A	rs6741819 C>T	109	48,62	40,37	11,01	0,53	100	0,312
SDF1	rs1029153 T>C	109	60,55	36,7	2,75	0,29	100	0,211
SDF1	rs10793538 A>T	109	93,58	6,42	0	0,73	100	0,032
SDF1	rs1801157 G>A	109	60,55	32,11	7,34	0,28	100	0,234
SDF1	rs2236533 G>A	109	66,97	30,28	2,75	0,75	100	0,179
SDF1	rs2236534 G>T	109	73,39	23,85	2,75	0,62	100	0,147
SDF1	rs2297629 G>C	109	98,17	1,83	0	0,92	100	0,009
SDF1	rs266087 G>A	108	37,04	46,3	16,67	0,72	99,08	0,398
SDF1	rs266092 T>A	109	90,83	9,17	0	0,62	100	0,046
SDF1	rs266093 G>C	109	37,61	43,12	19,27	0,26	100	0,408
SDF1	rs2839690 T>C	109	60,55	33,94	5,5	0,79	100	0,225
SDF1	rs3780891 G>A	109	77,06	22,02	0,92	0,62	100	0,119
SOX5	rs1464500 T>G	109	53,21	36,7	10,09	0,30	100	0,284
SREBP1	rs1108511 C>T	109	79,82	20,18	0	0,24	100	0,101
SREBP1	rs11868035 G>A	109	40,37	48,62	11,01	0,50	100	0,353
SREBP1	rs1889018 C>T	109	28,44	46,79	24,77	0,51	100	0,482
SREBP1	rs2297508 C>G	108	27,78	51,85	20,37	0,66	99,08	0,463
SREBP1	rs4925118 C>T	109	70,64	27,52	1,83	0,64	100	0,156
SREBP1	rs9902941 C>T	109	24,77	50,46	24,77	0,92	100	0,500
TCF7L2	rs12255372 G>T	109	45,87	39,45	14,68	0,19	100	0,344
TCF7L2	rs7903146 C>T	103	41,75	46,6	11,65	0,80	94,5	0,350
TNF	rs2244546 C>G	109	85,32	14,68	0	0,41	100	0,073
TNF	rs2244579 G>C	109	37,61	50,46	11,93	0,40	100	0,372
TNF	rs2518027 C>A	109	54,13	37,61	8,26	0,62	100	0,271
TNF	rs2523674 C>T	109	35,78	49,54	14,68	0,70	100	0,394
TNF	rs2523676 G>A	109	77,98	21,1	0,92	0,68	100	0,115
TNF	rs2844508 G>C	108	51,85	37,04	11,11	0,25	99,08	0,296
TNF	rs4713466 C>T	104	78,85	20,19	0,96	0,79	95,41	0,111

TNF α	rs1800629 G>A	109	85,32	12,84	1,83	0,11	100	0,083
TNF α	rs3093662 A>G	109	82,57	17,43	0	0,32	100	0,087
TNF α	rs361525 G>A	108	89,81	10,19	0	0,58	99,08	0,051
USF1	rs1556259 T>C	109	79,82	16,51	3,67	0,03	100	0,119
USF1	rs1556260 G>A	109	79,82	16,51	3,67	0,03	100	0,119
USF1	rs2073653 A>G	107	79,44	16,82	3,74	0,03	98,17	0,121
USF1	rs2516837 C>T	108	40,74	37,96	21,3	0,03	99,08	0,403
USF1	rs6686076 T>C	108	80,56	15,74	3,7	0,02	99,08	0,116

Table 2. Demographic, clinical and biochemical characteristics and LPV/r PK parameters of the studied population (N=109)

CHARACTERISTICS	VALUES Mean ± SD (range) or N (%)
Demographic	
Gender (male)	79 (72.47)
Age (years)	41 ± 9.14 (18-75)
Weight (kg)	67.1 ± 14.58 (41-122)
Height (cm)	169.07 ± 8.86 (150-189)
Body mass index (kg/m^2)	23.33 ± 4.01 (15.06-38.15)
Race (European)	106 (97.24)
Clinical and Biochemical	
RTV minimum concentration (mg/L)	1.03 ± 0.47 (0.38-2.87)
TB (mg/dL)	0.85 ± 0.55 (0.3-3.65)
TG (mg/dL)	212.94 ± 128.82 (75.5-854)
TC (mg/dL)	187.12 ± 45.22 (79.5-328)
cLDL (mg/dL)	97.78 ± 34.21 (21-184.5)
cHDL (mg/dL)	47.65 ± 17.65 (18-118)
cLDL/cHDL	2.24 ± 0.94 (0.65-5.1)
CD_{4+} (x 10^6/µL)	400.01 ± 219.97 (60.5-1219)
Plasma viral load detectable	451 (38.53)
PK parameters	
C^{ss}_{min} (mg/L)	8.23 ± 3 (2.74-19.62)
C^{ss}_{mean} (mg/L)	9.71 ± 2.98 (4.86-20.88)
C^{ss}_{max} (mg/L)	10.87 ± 3.02 (5.79-22.29)
CL/F (L/h/kg)	0.06 ± 0.02 (0.03-0.11)
V/F (L/kg)	1.82 ± 0.54 (0.6-4.52)

SD, standard deviation; RTV, ritonavir; TB, total bilirubin; TG, triglycerides; TC, total cholesterol; cLDL, low-density lipoprotein cholesterol; cHDL, high-density lipoprotein cholesterol ; cLDL/cHDL, low/high-density lipoprotein cholesterol ratio ; C^{ss}_{min}, minimum steady-state plasma concentration; C^{ss}_{mean}, mean steady-state plasma concentration; C^{ss}_{max}, maximum steady-state plasma concentration; CL/F, apparent oral clearance ; V/F, apparent distribution volume.

Table 3. Results from linear regression of each SNP on LPV/r PK parameters

GENE	SNP Genotype	Pharmacokinetic parameters*					
		C^{ss}_{min} (mg/L)	p-value	C^{ss}_{max} (mg/L)	p-value	CL/F (L/kg/h)	p-value
DRD3	rs1486012T>A		0.00009		0.00120		0.00044
	TT (n=40)	7.52		10.04		0.066	
	TA/AA (n=69)	8.64		11.35		0.059	
DRD3	rs963468G>A		0.00053		0.00382		0.00663
	GG (n=49)	7.75		10.29		0.065	
	GA/AA (n=60)	8.61		11.35		0.059	
TPMT	rs1142345A>G		0.02491		0.00568		0.00135
	AA (n=104)	8.20		10.84		0.062	
	AG (n=5)	8.86		11.55		0.050	
TPMT	rs1800460G>A		0.02523		0.02348		0.00284
	GG (n=104)	8.21		10.88		0.062	
	GA (n=4)	9.71		11.94		0.045	
CDH13	rs17216786C>A		0.07883		0.00982		0.03727
	CC (n=89)	8.55		11.25		0.060	
	CA/AA (n=20)	6.81		9.19		0.068	
MTTP	rs6532823C>A		0.03497		0.05778		0.00408
	CC (n=74)	8.40		11.09		0.060	
	CA/AA (n=35)	7.87		10.41		0.065	
RESISTIN	rs3219177C>T		0.01107		0.00343		0.02496
	CC (n=76)	8.13		10.75		0.063	
	CT/TT (n=32)	8.48		11.24		0.059	
SREBP1	rs2297508C>G		0.00929		0.01543		0.00482
	CC (n=30)	8.19		11.01		0.065	
	CG/GG (n=78)	8.24		10.83		0.061	
TNF-α	rs3093662A>G		0.08915		0.40258		0.00321
	AA (n=90)	8.21		10.90		0.063	
	AG (n=19)	8.33		10.75		0.057	

*Pharmacokinetic parameters were log-transformed before the analysis.

C^{ss}_{min}, minimum steady-state plasma concentration; C^{ss}_{max}, maximum steady-state plasma concentration; CL/F, apparent oral clearance.

Table 4. Results from longitudinal analysis of each SNP on CD_{4+} (A) and HIV viral load (B)

GENE	SNP	p-value	Estimate
ABCG2_BCRP	rs2231137	0.00788	4.57049
APOA	rs619054	0.00455	-3.20053
CDH13	rs17216786	0.00720	3.84724
SDF1	rs266087	0.00025	-4.12798

A

GENE	SNP	p-value	Estimate
IL6	rs2069837	0.00303	7.33
SDF1	rs266092	0.00052	11.02

B

Table 5. Results from longitudinal analysis of each SNP on lipids and total bilirubine levels

GENE	SNP	TC		cLDL		cHDL		cLDL/cHDL		TB	
		p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate
ABCC2_MRP2	rs3740066	0.02130	0.10866	0.00447	0.20871	0.33731	-0.06173	0.00950	0.23715	0.16303	-0.12503
CETP	rs11076174	0.24294	0.06552	0.01964	0.19497	0.16089	-0.10440	0.00238	0.30925	0.00207	-0.32548
CETP	rs11076175	0.30834	0.04695	0.05618	0.13323	0.05574	-0.11668	0.00782	0.22661	0.05263	-0.17158
CETP	rs11508026	0.33844	0.04501	0.87771	-0.01100	0.00758	0.16370	0.08724	-0.15364	0.25841	0.10373
CETP	rs9929488	0.38041	0.03884	0.04229	0.13604	0.10239	-0.09600	0.00927	0.21360	0.44149	-0.06612
CETP	rs9939224	0.51831	0.02913	0.05292	0.13149	0.07727	-0.10517	0.00989	0.21590	0.01889	-0.20333
LEP	rs1137100	0.30267	-0.04692	0.37337	-0.06190	0.00838	-0.15672	0.18793	0.11257	0.58390	0.04820
MCP-1	rs4586	0.00226	-0.13877	0.02819	-0.15498	0.58834	0.03348	0.06543	-0.16125	0.14191	0.13132

TC, total cholesterol; cLDL, low-density lipoprotein cholesterol; cHDL, high-density lipoprotein cholesterol ; cLDL/cHDL, low/high-density lipoprotein cholesterol ratio ; TB, total bilirubine.

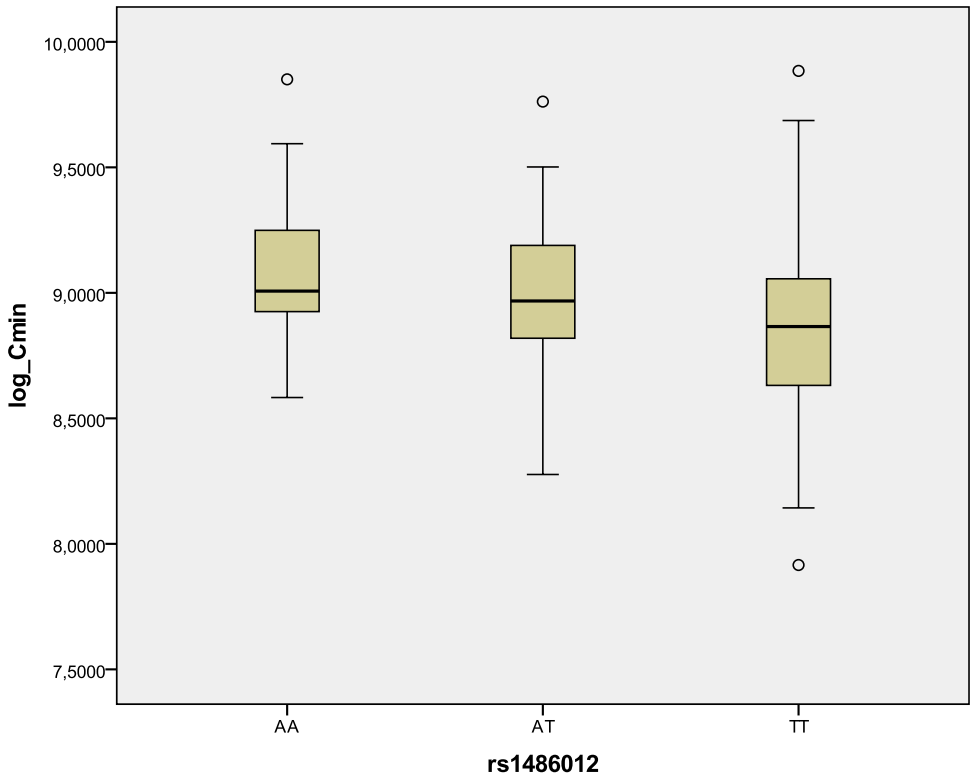

Figure 1. Box plot of minimun steady-state lopinavir/ritonavir concentrations log transformed derived from a dose of 400/100 mg twice daily in relation to DRD3 rs1486012 T>A genotypes.

POPULATION PHARMACOKINETIC/PHARMACOGENETIC MODEL OF LOPINAVIR/RITONAVIR IN HIV-INFECTED PATIENTS

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Para enviar a *Antimicrobial Agents and Chemotheraphy*

INTRODUCTION

Lopinavir (LPV) has demonstrated efficacy and safety in both naïve and protease inhibitors (PI)-experienced HIV-infected patients, therefore this drug remains as a recommended component of the combined antiretroviral therapy (cART) for certain patients at current clinical practice (DHHS, EACS and Gesida Guidelines). LPV suffers an extensive first-pass hepatic and intestinal metabolism, by the cytochrome P450 (CYP) 3A isoenzymes (Kumar, 1999), and the cellular efflux transport by P-glycoprotein (MDR-1) (Agarwal, 2007). Due to its low oral bioavailability, LPV needs to be coadministered with low dose of the booster ritonavir (RTV) which inhibits LPV first-pass effect, to achieve drug concentrations high enough to inhibit viral replication and allowing less frequent dosing (Sham, 1998; Olson, 2002; Kempf, 1997; Cooper, 2003; King 2004). Since 2001, LPV is the only PI coformulated with RTV.

Relationships between LPV plasma concentrations boosted with RTV (LPV/r) and efficacy or toxicity have been observed, thus achieving target concentrations is vital for the success of the cART containing this drug (Masquelier, 2002; Gutiérrez, 2003; Van der Leur, 2006; DHHS). Despite the known benefit of LPV/r, a considerable interindividual variability (IIV) in plasma concentrations and response is observed during the treatment with this drug (Moltó, 2006; Van der Leur, 2006). In this sense, the treatment with standard doses of LPV/r (400/100 mg twice daily [bid]) could lead to non-optimal plasma LPV/r trough concentrations in some patients, with the emergence of drug resistance and absence of virological response (Johnson, 2006; Back, 2002; Voigt, 2004) or toxicity (Gutiérrez, 2003; González de Requena, 2003; González-Requena, 2004).

Although variables such as age, gender, weight, body mass index, liver function, pregnancy and drug interactions have been identified to affect LPV/r plasma concentrations (Crawford 2010, Aspiroz 2011, ,Jullien 2006, Moltó 2008,Peng 2010, Bouillon-Pichault 2009), the pharmacokinetic (PK) IIV is still observed even after taking these covariates into consideration. Among the factors contributing to this unexplained PK variability, host genetic characteristics have been appointed as an important cause (Lubomirov 2011, Elens 2009, Owen 2008). Thus, the expression and functional activity of many proteins, such as CYP enzymes (Kumar 1999), drug transporters (Agarwal 2007, Hartkorn 2010) and some receptors (Kliewer 2003, Peschl 1978) involved in absorption-distribution-metabolism-excretion (ADME) process, may contribute to these observed interpatient differences in LPV/r concentrations, although they have not been well identified yet.

Recently, pharmacogenetic (PG) studies showed an influence on L PV/r PK of some single nucleotide polymorphisms (SNPs) in genes encoding for CYP3A isoenzymes, multidrug resistance protein-2 (MRP-2) and organic anion transporting polypeptides (OATP or SLCO) (Lubomirov 2010, G upta 2008, Elens 2009, Hartkorn 2010, Kohlrausch 2010). The alleles of the SLCO1B1 gene, SLCO1B1*4 and SLCO1B1*5, have been identified as responsible for the increased and reduced apparent oral clearance of LPV/r (CL/F), respectively (Lubomirov 2011). However, other studies of SNPs in ABCB1 gene, encoding for MDR-1 which is a known LPV/r protein transport, have not found any relevant association (Elens 2009, Gupta 2008, Ma 2007, Winzer 2003). Also, the results about the impact of SNPs in CYP3A on LPV/r kinetics still remains controversial (Lubomirov 2010, Elens 2009, Estrela 2008, Gupta 2008). In this sense, it is important to remind that RTV is a potent inhibitor of MDR-1, CYP3A4 and

CYP3A5 (Olson 2002, Kempf 1997, Cooper 2003), mechanisms of the "boosting" effect, and could explain that these SNPs do not influence on PIs PK (Owen 2008). Additionally, the small sample size and the lack of well designed studies are some inconveniences of the PG research carried out to date to demonstrate conclusive associations.

Therefore, it is important to increase our knowledge about the influence of the genetic factors on PK behaviour of LPV/r, which would be of critical importance to better characterize the ADME process and thus PK phenotypes (Van der Leur 2006). Few population PK analyses on LPV/r have been reported in HIV-infected population using nonlinear mixed effects modeling (NONMEM) (Moltó 2008, Crommentuyn 2005, Bouillon-Pichault 2009, Lubomirov 2010, Aspiroz 2011) and only one of them has integrated PG information (Lubomirov 2010). Consequently, the objective of this study is to identify associations between LPV/r PK parameters and 113 SNPs in genes encoding for ADME involved proteins as well as clinical, demographic and treatment characteristics of the patients and to develop and validate a population PK/PG model using NONMEM in a HIV-infected European population. The availability of this population PK/PG model of LPV/r would allow its implementation in clinical PK software and its application in dose individualization in clinical practice using a Bayesian approach (Moltó 2008, Crommentuyn 2005).

MATERIAL AND METHODS

Study Population and Sampling Design

The population PK/PG analysis was conducted on HIV-infected patients treated with LPV/r, from the outpatient pharmacy of the University Hospital of Salamanca (Spain).

Some of these patients had been included in a previous PK study with collected data from October 2006 to October 2008 (Aspiroz 2011). This study included updated PK data until January 2011, as well as PG information and other biochemical information. The demographic and clinical characteristics of the analyzed patients are shown in Table 1.

The inclusion criteria were as follows: confirmed HIV infection; treatment initiated with standard LPV/r dose at least one month before their inclusion in the study; adherence to the treatment regimen over than 90%; aged≥ 18 years and no concomitant use of non-antiretroviral (ARV) drugs known to be inductors or inhibitors of LPV/r. All patients had normal hepatic and renal function when treatment was started. All patients were initially administered 400/100 mg oral LPV/r twice daily in combination with other ARVs as part of their cART. Approximately 13% of patients required dose adjustments (range 200/50-500/125 mg bid) to achieve recommended LPV/r trough concentrations (1 and 4 m g/L for naïve and pre-treated patients, respectively [Masquelier 2002, Gutiérrez 2003, Van der Leur 2006]). The study was subjected to approval by the ethics committee of the University Hospital of Salamanca and all patients included in this study provided written informed consent for genetic testing.

The patients came to the outpatient pharmacy to pick up t heir cART monthly. All of them were included in a therapeutic drug monitoring (TDM) program, and plasma samples for LPV/r concentration assays and biochemical tests were drawn periodically at 3 t o 6 month intervals during follow-up visits to the hospital. One of these blood samples was used to genetic analysis. Individual patient information was carefully recorded, including: gender, age, weight, height, dose history, time of last dose,

sampling time, hepatitis C virus (HCV) coinfection and concomitant treatment (ARVs and other drugs). Treatment adherence was measured according to six-monthly dispensing records and a Simplified Medication Adherence Questionnaire (Knobel, 2002); when this questionnaire provided an adherence over than 90%, a coefficient of variation (CV) of the mean LPV/r plasma concentration/dose ratio less than 30% was used as additional criterion according to previously observed intrapatient variability (Csajka, 2003). Data concerning biochemical parameters related to lipid profile (triglycerides [TG], total cholesterol [TC], low-density lipoprotein cholesterol [cLDL], high-density lipoprotein cholesterol [cHDL], coefficient cLDL/cHDL) and liver function (total bilirubin [TB]) were also collected.

Blood Sampling and Drug Assay

Most blood samples were collected at the end of the dosage interval, between 10 and 14 hours after LPV/r administration, under steady-state conditions (unchanged dose at least one month before).

Blood samples (5 mL) were collected and plasma was isolated by centrifugation at 3000 g. Samples were stored at−20 °C (following virus inactivation in a water bath at 60°C for 60 minutes) until analysis. LPV/r concentrations were measured by high-performance liquid chromatography (Waters, Milford, USA) with ultraviolet detection at 215 nm, following solid phase extraction using a GX-271 ASPEC (Gilson, Villiers le Bel, France). This method was validated over a LPV concentration range of 1 to 10 mg/L, using 600 µL of plasma. Recovery of LPV from human plasma was 106.4%. Intra- and inter-day CV precisions were consistently < 3 .5% for all internal quality controls (2.0, 6.0 and 10.0 mg/L). The quantification limit was 0.50 mg/L and absence

of interference from the 21 drugs most often used in HIV+ patients was confirmed. Our analysis laboratory successfully participates in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection (a Dutch association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology [KKGT]).

SNP Selection

Genetic polymorphisms were selected based on three main criteria: (i) SNPs with identified or presumed functions in genes encoding for enzymes and transporters of LPV/r; (ii) the SNPs chosen were either functional SNPs (based on potential protein changes) or in linkage disequilibrium with a functional one; or (iii) SNPs which had been reported by other groups on public databases (CYP alleles: www.cypalleles.ki.se, for the metabolizing enzymes, and NCBI: www.ncbi.nlm.nih.gov/SNP/ and SYSNPs: http://www.sysnps.org, for the other genes). From NCBI and SYSNPs databases, were selected the SNPs with a high minor allele frequency (MAF) and those tagSNPs which were representative of some genes.

A total of 113 SNPs were chosen for the analysis using the above criteria. The SNPs investigated and their frequencies in the patients are shown in Table 2. According to a multivariate association analysis using PASW Statistics 18 (IBM SPSS Statistics) and R for Windows (González, 2007) between SNPs and the PK parameters of LPV/r, those 15 SNPs showing the lowest probabilities were selected to be included in the PK/PG analysis (data not shown).

Genotyping Analysis

All genotyping experiments were conducted at the Spanish National Genotyping Center (CeGen). Genomic DNA was isolated automatically from 5 mL whole human blood with magnetic bead technology using the Chemagic Magnetic Separation Module I and the Chemagic DNA kit, according to the manufacturer's instructions (Chemagen AG, Baesweiler, Germany).

Genotyping was performed using a MassArray platform provided by Sequenom. This method involves multiplex PCR amplification of up to 7 SNPs. Excess nucleotides are removed by treatment with shrimp alkaline phosphatase, and multiplex primer extension is performed using a mix of deoxy- and dideoxynucleotides, so that products of different masses are obtained for each allele of each SNP. Resulting products are resolved by mass spectrometry (MALDI-TOF).

Several quality controls were carried out in the genotyping analysis. Firstly, three controls of Centre d'Etude du Polymorphisme Humain (CEPH) samples (preference of mother, father and son) as positive controls were included in each plate for all the SNPs genotyped through MALDI-TOF. The genotype of all this samples was known as they are reported in the HapMap database (www.hapmap.org). Additionally, two non-template controls were included on each plate, as well as 20% duplicate samples, whose allelic frequencies for Caucasian population were confirmed according to the NCBI database. Genotyping data were filtered using the genotype call rate (> 90% completeness), the Hardy-Weinberg equilibrium (HWE) test (p-value > 0.001) and a minor allele frequency (MAF) criterion (> 1%) in order to ensure adequate quality of the PG data.

Population Pharmacokinetic/Pharmacogenetic Model Development

A population PK/PG model of LPV/r was built using NONMEM (version VII; double precision, level 2.0) (Beal 1989). The first-order conditional estimation method was used in conjunction with Laplace approximation for all models tested during model development and the concentrations were fitted on a linear scale.

A one-compartment kinetic model with first-order absorption and elimination (specified to NONMEM by the ADVAN2 and TRANS2 routines), was assumed. Therefore, the estimated PK parameter was the CL/F, because values of the distribution volume (V/F), absorption rate constant (Ka) and the absorption lag time (ALAG) were fixed to 130 L, 1.9 h^{-1} and 1.78 h according to the previous PK model developed by our group (Aspiroz 2011). Both additive and exponential error models were tested to explain inter-individual and residual variability.

To elucidate the preliminary relationships between the individual PK parameters and analyzed covariates, a graphical approach for the exploratory data analysis and the stepwise generalized additive model (GAM) implemented in Xpose 4.3.2 was used (Jonsson, 1999). Those covariates selected as t he most potentially important were incorporated in a stepwise manner into the basic model to develop the intermediate and full models.

Quantitative covariates (age, total body weight [TBW], body mass index [BMI], RTV trough concentration [RTC], TB, TC, TG, cLDL, cHDL and cLDL/cHDL) were included in both linear and nonlinear way. Discrete covariates (gender, HCV coinfection and concomitant treatment with other ARVs) were tested in the model as

binary variables (0 or 1). With respect to PG covariates, also took values of 0 or 1 to indicate the absence or presence of the rare allele. All discrete covariates were examined using a multiplicative model, in order to obtain the fractional increase or decrease in the associated PK parameter.

The criteria for retention of a particular covariate in the model were as follows: (i) the objective function value difference (DOFV) between two hierarchical models must be at least 3.84 (degrees of freedom [df] = 1) in order to achieve the desired level of statistical significance ($p < 0.05$); (ii) reduction in unexplained IIV for the associated PK parameter; (iii) randomly distributed weighted residuals (WRES) and individual weighted residuals (IWRES); (iv) a closer relationship between predicted and observed concentrations; (v) the 95% confidence interval (CI) of the covariate effect must exclude zero (or unit for discrete covariates); and (vi) the standard errors (SE) should not take values representing more than 25% and 50% of the value of estimated fixed and random parameters, respectively.

In addition, for a covariate to be considered as clinically relevant, a change in the typical population PK parameter estimate of ± 20% was required.

Model Qualification

Goodness of fit was evaluated by visual inspection of diagnostic scatter plots, including observed and predicted concentrations *versus* time, WRES *versus* time and residual *versus* predicted concentrations. Using the basic and final covariate models both visual predictive check (VPC) and numerical predictive check (NPC) were performed by simulating 1000 subjects to assess the predictive performance of the model.

Calculations were made from a package implemented in Xpose 4.3.2 (Jonsson 1999). Additionally, normalized prediction distribution error (NPDE) was computed, as described by Brendel et al. 2006, from 1000 replicates for each observed data point generated by NONMEM for the patient groups that received different doses according to the covariates found to affect CL/F in a relevant way. Wilcoxon signed rank test and Fisher test were used to test whether the mean and the variance were significantly different from 0 and 1, respectively and the Kolmogorov-Smirnov test to probe if NPDE followed a N[0, 1] distribution (Comets 2008). Complementary statistical analyses were performed using SPSS (version 19, SPSS, Inc., Chicago, IL).

RESULTS

A total database of 693 LPV/r plasma concentrations from 109 patients were available for population PK/PG modeling. The mean number of LPV/r plasma concentrations per patient was 6.35 ± 3.2 (1-13) and as can be observed in Table 1, it shows a high interindividual drug disposition variability as the mean measured concentration was 8.3 ± 3.15 mg/L within a range from 1.06 to 23.6 mg/L. One compartmental model with first order absorption and elimination fitted adequately LPV/r data concentrations.

In the base model, without covariates, the estimated value for CL/F was 3.96 L/h (SE = 0.00997; which suppose 0.25% of the estimated value for this parameter) and the observed IIV, well described by proportional error model was 26.65%. Residual variability, also according to a proportional error model, was 20.47%.

Graphical exploratory analysis of the relationship between individual Bayesian CL/F value estimated by NONMEM (using the POSTHOC option) and non-genetic

covariates by GAM, revealed that BMI, RTC, cHDL and concomitant treatment with tenofovir (TFV) showed some influence on CL/F. Table 3 summarizes the most significant models evaluated, showing the main covariates with influence on CL/F and the main parameters used in their discrimination. As can be observed, BMI, cHDL and RTC had a statistically significant effect on this PK parameter, which led to a reduction in OFV of 278.242 (intermediate model) ($p < 0.05$) as well as in the IIV and the residual variability (Table 3, model 2). The inclusion of TFV in the model produced a significant reduction on the OFV but the effect was lower than 20%, so it was not retained in the final model. In regard to the others ARVs analyzed, the LPV/r plasma concentration samples containing efavirenz (EFV), atazanavir (ATV) and saquinavir (SQV) were less than 1.5% of the total samples, so no correlation was explored between these drugs coadministration and LPV/r PK parameters.

With respect to PG covariates, these 15 SNPs were selected to be included in the PK/PG model development: CYP3A5 rs28371764, CYP3A4_1461ins, CYP3A rs6945984, CYP1A2_163CA, MDR1_G2677AT, MRP-2 rs8187707 and 717620, 5HT2C_CYS23SER, DRD3 rs1486012, SLCO1B1 rs11045819, rs4149032 and rs4149056, SLCO1B3 rs4149117, SLCO1A2 rs10841798 and rs11837182, as they showed the most important specific influence on the CL/F or the LPV/r minimum steady-state plasma concentrations (C^{ss}_{min}) in the preliminary exploratory statistic analysis. They were added one by one independently on the intermediate model and those showing a significant influence were explored jointly on the intermediate model (Table 3). The following PG covariates, although showed a significant reduction in OFV, its effect on CL/F was less than 15%: rs8187707 (13%), rs4149032 (12%), and rs11045819 (11%), which is clearly lower than the 20% assumed to be covariates with

clinical relevance. Only the SNPs CYP3A5 rs28371764, CYP3A4_1461ins and CYP3A rs6945984 showed a change in CL/F upper to this value and its inclusion in the model resulted in significantly reduction in OFV and in IIV of CL/F.

Figure 1 shows some significant relationships found between post hoc Bayesian LPV/r CL/F and different demographic, biochemical and PG covariates investigated using Xpose.

In conclusion, the final model adopted for CL/F was as follows:

$$CL/F = (\theta_1 \cdot BMI + \theta_5 \cdot cHDL) \cdot \theta_6^{RTC} \cdot \theta_7^{rs28371764[C/T]} \cdot \theta_8^{rs6945984[C/C]} \cdot \theta_9^{CYP3A4[1461insA/del]}$$

where θ_1 through θ_9 were fixed parameters (θ_2, θ_3 and θ_4 were the coefficients for V/F, Ka and ALAG, respectively), rs28371764 [C/T], rs6945984 [C/C] and CYP3A4 [1461insA/del] were assigned values 0 or 1 according to the absence or presence of these genotypes, respectively.

Residual variability decreased 30.07% in the final model in comparison with the base model (variance of 0.0293 *versus* 0.0419). Table 4 shows the values of fixed and random parameters estimated in the final population PK/PG model proposed for LPV/r.

In addition, examination of the scatter plot of WRES *versus* predicted concentrations obtained from the final model revealed a significant improvement in pattern (random distribution) with respect to the base model. The OFV decreased with a difference of 324.391 (df = 5), p = 2.817E-68, which is higher than the critical value of χ^2 of 11.070 and consequently fulfills the statistical required criteria ($p < 0.05$). Figure 2 shows scatter plots of measured *versus* predicted LPV/r concentrations and WRES *versus*

predicted concentrations by the base and the final models. These plots show an improvement in fit for the final model, reflected as tighter scatter around the identity line. Furthermore, the linear regression correlation coefficient between the observed *versus* the fitted population concentrations was 0.693 for the final model, which is significantly better than the 0.001 obtained for the base model.

Figure 3A shows plots of the time course of the observations and prediction interval (PI) for the simulated values for the base and final models which provides a VPC; and Figure 3B graphically shows the results obtained from the NPC. The percentage of observations above and below 90% PI was 4.47% in both cases for base model and 4.04% and 5.39%, respectively, for the final model.

The results obtained in the model qualification support the final model proposed for a simulated population, as described in the Material and Methods section. Figure 4 shows the quantile-quatile plot of the LPV/r NPDE distribution. As can be observed, the assumption of N[0,1] distribution appears to be reasonable since deviations from the identity line shows minimal departures from the expected distribution. Mean and variance of these NPDE were found to be 0.04018 and 1.00338, close to the expected values of 0 and 1, respectively. In fact the Kolmogorov-Smirnov test showed that NPDE followed an N[0,1] distribution (p-value = 0.673); hence, the hypothesis tested can be accepted and the model proposed can be considered adequate.

DISCUSSION

In general for the PIs most frequently used at the moment, ATV and darunavir, few PK models in HIV-infected patients have been published and rarely include PG information

(Colombo 2006, Solas 2008, Dickinson 2009, Dailly 2009, Schipani 2010). However, for SQV, indinavir and nelfinavir, more experienced drugs, some PK/PG information can be seen in the literature which showed certain conclusive results useful in the dosage optimization with these drugs (Mouly 2005, Bertrand 2009, Anderson 2006, Haas 2005). In this sense, EFV belonging to another ARV family, has been the most widely studied, showing a significant influence of the CYP2B6 516G>T on its CL/F, which advice dosage adjustment in order to achieve a safe and effective treatment. These results suggest the importance of the knowledge of the influence of some SNPs in the PK behavior of the ARVs.

A one-compartment linear PK model with first order elimination and absorption, including a lag-time, adequately described the LPV/r plasma concentration data. The sparse data used in this analysis, obtained usually at the end of the dosage interval made necessary to fix some PK parameters in order to get a good estimation of CL/F and its variability. Thus V/F, Ka and lag time were fixed to 130 L, 0.9 h^{-1} and 1.78 h, respectively, according to the values obtained from other previous population PK model developed by our group (Aspiroz 2011). This model included full PK profiles (rich data) in the 40% of patients. A mean value of 3.96 L/h was obtained for CL/F in the base model without any covariates (Table 3), similar to our previous study and slightly lower than those observed by other researches (4.10-5.07 L/h), (Moltó 2008, Bouillon-Pichault 2009, Lubomirov 2010, Crommentuyn 2005).

In this study, RTC, BMI, cHDL and TFV coadministration were the demographic, biochemical and treatment covariates selected by GAM as significantly related to CL/F, although the last one was not included in the final model. The magnitude of RTC was

the covariate explaining the most part of the IIV of the CL/F as can be expected, due to the known inhibition of the intestinal and hepatic CYP3A-dependent metabolism and the P-glycoprotein-mediated transport (Kumar 1999). The close relationship previously demonstrated between the area under the curve of RTV and RTC (Aspiroz 2011) allowed the use of the last one, easier to measure, as predictive covariate of CL/F. However, RTV boosting effect could hide the real influence of genetic polymorphisms of genes responsible for metabolism and transport of LPV.

Previous PK population studies had also included covariates related with body size on CL/F, such as BMI (Aspiroz 2011, Boffito 2008) or TBW (Lubomirov 2010, Bouillon-Pichault 2009, Ribera 2004), although some of them did not found any influence (Moltó 2008, Crommentuyn 2004, Crommentuyn 2005). Although discrepancies exist concerning the most adequate covariate to describe body size, in this study the best model fit was obtained with BMI instead of TBW, which was included in the final regression model of CL/F. For a standard patient with mean values of BMI, cHDL and RTC of 23.25 kg/m^2, 46.8 mg/dL and 1.05 mg/L, respectively, the CL/F estimated was 3.93 L/h. However, for individuals of our population with the lowest BMI value (15.06 kg/m^2) or the highest one (38.75 kg/m^2), this PK parameter would be 2.69 or 6.28 L/h, respectively. As can be observed, the CL/F changes significantly between individuals with extreme values of BMI, which reflects the relevance of this covariate in the elimination of LPV/r.

Despite being a controversial topic, in our study gender did not improve the fit of data and it was not include in the final model, which is consistent with the results of the most of the LPV/r population studies (Aspiroz 2011, Moltó 2008, Crommentuyn 2005,

Bouillon-Pichault 2009, Crommentuyn 2004, B offito 2008, R ibera 2004, Ofotokun 2007, Robbins 2008). Some authors found higher CL/F in male than in female patients, but it was associated with the lower TBW shown in that last population group (Lubomirov 2010, Burger 2002).

Among the biochemical covariates analyzed in this study, only cHDL showed a significant relationship with the LPV/r PK parameter. Several reasons could explain this finding, not previously reported as far as we know. Although it is widely known that LPV/r is highly bound to albumin and α_1-acid glycoprotein (EMA), less known is its binding to plasma lipoproteins which could happen due to the lipophilic character of LPV/r (EMA). The HIV-infected patients often have an altered lipid profile due to the HIV replication, chronic hepatitis coinfections and the cART which could cause significant differences in the lipoprotein concentrations (Lemaire 1986, Piafsky 1980). Indeed, the mean TG and TC values of our population were 220.10 ± 157.93 and 192.04 ± 46.92 mg/dL respectively, higher than the upper limits of normal (200 and 150 mg/dL, respectively). Therefore, as cholesterol binds to HDL lipoprotein, correlations between unbound LPV/r fraction in plasma and lipoproteins concentrations would be expected in this scenery (Evans 1992). Because the clearance of the drug depends on the drug unbound fraction, the effect of a compound able to cause a lipoprotein-binding displacement, such as cHDL in this clinical situation, could explain the increase found in CL/F with increments in this biochemical parameter (Barrer). As can be seen, the patient with the highest cHDL value (126 mg/dL) analyzed, presents an estimate CL/F of 4.62 L/h, significantly higher than the mean standard value of this PK parameter (3.93 L/h). Actually, information concerning the concentration of albumin and α_1-acid glycoprotein is rarely incorporated to the PK models (Moltó 2008), and lipid levels have

never been considered, which could be interesting for future researches. In fact, this is the first evidence of the influence of cHDL on the LPV/r elimination which seems to be clinically relevant.

Although none other clinical and biochemical covariates could be included in the developed model, HCV coinfection and parameters associated with chronic liver disease have shown influence on CL/F in other researches (Moltó 2008, Ribera2004). These results seem coherent because this drug is mainly eliminated through hepatic metabolism and is highly bound to plasma proteins (Kumar 1999, EMA). In our study it was not possible to analyze serum transaminase levels or the extent of liver fibrosis as the authors who found a correlation did, and only TB and HCV coinfection were evaluated as covariates concerning the liver function.

The concomitant treatment with TFV, significantly elevated among the studied population (72.66%), led to slightly higher concentrations of LPV/r due to a reduction in its CL/F (factor of 0.91). Although the inclusion of this covariate on CL/F significantly improved the fit of the final model (DOFV = 10.540 with respect to the intermediate model), it hardly reduced the variability of this PK parameter (20.10% *versus* 19.75%). Besides, the influence was less than 20%, which did not fulfill the previously defined criteria to maintain the covariates in the model and thus TFV coadministration was eliminated from the population model. However, this trend could be justified by the fact that those patients with the highest RTC had TFV as a component of their cART, being the elevated RTC responsible for the decrease in CL/F instead of the TFV coadministration (data not shown). Indeed, the influence of this covariate could not be demonstrated in some PK studies published in the literature

(Moltó 2006, Crommentuyn 2005, Kearney 2003, Kearney 2006); moreover, the opposite trend (lower LPV/r exposure) has been observed by other authors (Ofotokun 2008, Flaherty 2001, Scarsi 2004, Breilh 2004, Aspiroz 2011) although the influence neither was clinically relevant.

Once the influence of demographic and biochemical characteristics as w ell as concomitant drugs administered were analyzed (intermediate model: Table 3, model 2), the PG covariates were tested. Only 15 SNPs from the 81 available for the analysis were selected using a u nivariate statistical test whose influence on Bayesian CL/F was preliminarly analyzed using GAM. Although the MAF of the CYP3A4_1461ins was not >1%, an exception was considerate to explore this SNP in the analysis due to the importance of this gene on LPV/r metabolism (Kumar 1999). Figure 1 shows the most significant relationship found between the main PG covariates and the CL/F.

The CYP3A has been considered as the most important metabolizing enzyme system of LPV/r (Kumar 1999), but the contribution of CYP3A4 and CYP3A5 has not been well described to date. In this study, the SNP CYP3A4_1461ins, which characterizes the CYP3A4*20 allele, showed the strongest influence on CL/F. This allele cause a premature stop codon yielding a truncated protein non functional (Westlind-Johnsson, 2006) which could explain the reduction in the CL/F by a factor of 0.302. Only one of the patients genotyped presented this polymorphism which compromises the reliability of this result. However to have patients with the presence of this genetic variant is interesting because its low frequency among whites is 0.06% (Westlind-Johnsson, 2006). The significant reduction in OFV ($p << 0.01$) allowed to maintain this covariate in the final model. Indeed the C^{ss}_{min} of LPV/r of this patient with CYP3A4_1461insA

heterozygous genotype were much higher than that found in the remaining population even after a dose reduction to 300/75 mg bid, which were over than the C^{ss}_{min} of 1 or 4 mg/L, recommended for naïve and pretreated patients, respectively (Masquelier 2002, Gutiérrez 2003, Van der Leur 2006). Therefore, subjects with this genotype might be susceptible to side effects during the cART. Because of its potential clinical relevance and the low frequency of the SNP among Europeans, further studies are necessaries to confirm this result.

The other member of this subfamily is polymorphically expressed and the active enzyme is codified by the wild-type CYP3A5*1 allele. Only people with at least one CYP3A5*1 allele express large amounts of CYP3A5 (Kuelh 2001, Daly 2006). Because CYP3A5 may represent up to 50% of total CYP3A system in individuals with the presence of this allele CYP3A5*1, differences in its expression have been suggested to be potentially responsible for the IIV in the ADME process of SQV, another PI, and some immunosupresors mainly metabolized by the CYP3A (Kuehl 2001, Mouly 2005, Josephson 2007, Haufroid 2006, Le Meur 2006). In this study, the presence of the rare allelic variant of rs28371764, which defines a subtype of the allele above mentioned (CYP3A5*1C), was also able to explain part of the IIV of the CL/F (variance of CL/F for intermediate model was 0.0404 *versus* 0.0377 for the model considering this SNP); this result has not been previously reported mainly because it has never been analyzed. The patients carrying one of the CYP3A5*1C alleles (9%) demonstrated an increment of upper than 25% in CL/F, thus showing a mean value of 4.95 L/h *versus* the 3.93 L/h estimated in non-expressors of this allele (Figure 1D). Because this SNP (-74 C>T) is located in the promoter region of the gene (5PRIME_UTR CYP3A5), the T rare allele seems to induce a great expression of the CYP3A5, maybe in liver and in other

extrahepatic tissues, which could to explain the larger CL/F found; besides this polymorphism could be responsible of a decreased bioavailability or a combination of both effects. In clinical practice, the significant impact of the genetic variants of the CYP3A5 has been considered for other drugs such as saquinavir, tacrolimus and sirolimus (Mouly 2005, Josephson 2007, H aufroid2006, Le Meur 2006). Thus, our result concerning the influence of the CYP3A5*1C on CL/F of LPV/r would need to be confirmed in a larger number of patients including also homozygote carriers of the genetic variation, in order to give more consistency to this hypothesis about this novel association before its consideration in the optimization of the LPV/r-based therapy.

Although it is known that patients with CYP3A5*3 and CYP3A5*6 alleles express a non-functional CYP3A5 isoenzyme, the published studies about the influence of these alleles on t he PK of LPV/r, have not observed any significant influence to date (Rakhamanina 2011, Estrella 2008, Elens 2010). They ascribed the lack of this influence to the inhibition of the CYP3A5 caused by R TV (Rakhamanina 2011). Surprisingly, there is only one study carried out in African-American population, where a weak association was observed between the presence of the CYP3A5*3 and lower C^{ss}_{min} of LPV/r (Gupta 2008). However, this result should be interpreted with caution as only one patient was homozygous for the *3 a llele; moreover, the function and regulation of hepatic metabolic enzymes could be altered in severe chronic kidney disease, thereby resulting in lower concentrations of LPV/r (Gupta 2008). Our population was monomorphic for the SNPs that define the alleles CYP3A5*3K and *6. This fact could justify partially that these patients have not lost the activity of the CYP3A5 as can be deduced of the mean estimated CL/F, in spite of all the SNPs that are reported to describe this gene could not be completely genotyped.

Another of the most significant findings of the present study was the significant decrease of 53% in the CL/F when the tagSNP rs6945984 (situated in a linkage disequilibrium block that includes CYP3A4, 3A5 and 3A7) was present in homozygosis. In spite of the low presence of this genetic variation among the studied population, the significant reduction of the OFV (Table 3, model 7) as well as the contribution to the IIV of the estimated PK parameter, allowed the inclusion of the presence of the rare allelic variant of this SNP in homozygosis as a covariate in our final PK/PG model. Lubomirov et al. 2010, in a well designed PK/PG study found a decrease of similar magnitude on the CL/F associated to this SNP in homozygous, but also in heterozygous patients, being included in their final PK/PG model.

The remaining SNPs in other CYPs analyzed did not show any influence on the PK of LPV/r in agreement with previously studies where these SNPs were explored (Elens 2009, Estrela 2008, Gupta 2008). With respect to the other SNPs in candidate genes, although none of the remaining SNPs analyzed showed an enough significant influence to be included in the final model (> 20%), it is noteworthy to comment some of the results obtained.

The studies analyzing the influence on the PK of the LPV/r of polymorphisms in the ABCB1 gene, which encodes for the MDR-1, have not shown conclusive results (Elens 2009, Gupta 2008, Winzer 2003, Ma 2007). In this study, only the ABCB1 2677 G>T/A was tested in the final model because it was selected in the preliminary statistical analysis; however, it did not demonstrate any important influence in spite of it has been related with an alteration of the transporter activity in vitro (Kim 2001). The remaining

polymorphisms in ABCB1 gene were not tested in the final PK/PG model due to the lack of significant influence in the preliminary analysis which was in concordance with other investigations concerning this transporter (Elens 2009, Gupta 2008, Winzer 2003, Ma 2007).

LPV and RTV have been identified as substrates of the efflux transporter MRP-2, encoded by the ABCC2 gene, which is expressed on the apical domain of hepatocytes, enterocytes, renal proximal tubule cells and leucocytes (Agarwal 2007, Janneh 2007, Oselin 2003). Besides RTV is also an inducer of this transporter (Gutmann 1999, Huisman 2002, Dussault 2001). In relation to the SNP rs8187707, a statistically significant association between the polymorphism and the CL/F was found, showing that the individuals with heterozygous genotype had a 21% greater elimination of LPV/r than the wild-type ones (Figure 1H). However, when the SNP was added to the model considering the rs28371764, its influence was reduced to 13% (Table 3, model 5). In this sense, for the rs8187710, located in the same region of the gene, it was hypothesized that even if the rare variant allele might induce a higher surface expression of the protein (Meier 2006), this slight difference is not likely to compensate for the impaired activity of the MRP-2-mediated transport caused by this SNP (Elens 2011). Because of this, the accumulation of LPV/r in the hepatocytes could lead to an increased LPV/r metabolism, which may justify the tendency that we observed (Elens 2011). However, it did not finally fulfill the required statistical criteria to be included in the final model. Although Elens et al. 2009 neither found influence of the SNP rs8187710 on LPV/r plasma concentrations, a higher accumulation of LPV/r in peripheral blood mononuclear cells of HIV-infected patients was observed, that was associated with a lower MRP-2-mediated LPV/r cellular efflux.

Regarding to other SNP in the same ABCC2 gene, rs717620 (-24 C>T), the results are also controversial as it was initially associated with a reduced expression of the protein (Haenisch 2007, Haenisch 2008), while a most recent study did not found this alteration (Zhang 2010). Regarding the LPV/r PK, the homozygous carriers of the SNP showed a reduction of 40% in the CL/F which could be explained by a lower expression of MRP-2 responsible for an increase of LPV/r concentrations (Lubomirov 2010). However, in our study, although 7.4% and 30.1% of the genotyped population were homozygous and heterozygous for the rare allele, respectively, it was not observed any effect on LPV/r kinetic behavior which is in agreement with another research (Elens 2009). These results support the existence of controversy in relation to the protein expression, which may be explained by the difficulty in quantifying protein levels in vivo due to the numerous parameters that may affect ABCC2 expression levels.

Concerning the OATP, the SLCO1A2 gene was widely analyzed (Table 2) while only some candidate SNPs of the other SLCO genes were studied because in recent publications some of they explained part of the LPV/r PK variability (Lubomirov 2010, Hartkoorn 2010, Kohlraus 2009). In contrast with the rest of the LPV/r transporter proteins previously mentioned, RTV is not an inhibitor of SLCO1B1 (Hartkoorn 2010), so any hiding effect on PK of LPV/r is expected in the analysis regarding this gene. Lubomirov et al. 2010 identified the rs11045819 (SLCO1B1*4) as the most important SNP contributing to the IIV of CL/F in a population PK/PG model recently published (Lubomirov 2010). This genetic variation is related with an increase in the LPV/r transport activity into the hepatocytes, that caused nearly a double mean value of the CL/F in patients with *4/*4 genotype (Lubomirov 2010). In the present study, although

its inclusion in the final model produced a significantly decrease on OFV, the influence did not show clinical relevance (Table 3, model 10) which is in agreement with the study of Kohlraus et al. 2009.

The presence of other SNP, the rs4149056 (SLCO1B1*5), represented in 26.6% of our population, showed a 15% reduction in CL/F in patients with the genetic variation in homozygosis (and lower than 4% reduction in heterozygous) (Figure 1I). Because the influence was lower than the 20% required and the decrease in OFV was not significative, it was not included in the final model. A reduced CL/F was observed also by Lubomirov et al. 2010 in the same magnitude of 15%, and this tendency was also observed by other authors who found higher LPV/r plasma concentrations (Hartkoorn 2010, Kohlraus 2009). This SNP (521 T>C) has been associated with an impaired uptake of LPV/r by the hepatocytes (Kameyama 2005, Niemí 2007), thus explains this reduction in CL/F and the consequently increased plasma concentrations (Lubomirov 2010, Kohlraus 2009). However, Elens et al. 2009 did not observe this influence.

Among the other SNPs of the SLCO genes, also studied by other authors, the SLCO1B1 rs4149032 was selected to test its influence on CL/F in the final model because it demonstrated an increment on this PK parameter of 15% and 12%, in heterozygosis and homozygosis, respectively. Although this significant trend is in agreement with other analysis (Hartkoorn 2010), the clinical relevance of the SNP was not enough to be incorporated to the final model, fact also reported by other authors (Lubomirov 2010). In this latter study, it was emphasized the clinical importance of this association, due to the phenotype caused by this SNP which may predispose patients to an increased risk of acquisition of resistance (Hartkoorn 2010).

The SLCO1A2 gene was explored thoroughly due to the recent importance reported of these transporters in relation with LPV/r and its poor characterization to date (Hartkoorn 2010, Kohlraus 2009, Lubomirov 2010). From all the tagSNPs tested, only rs10841798 revealed a slightly relationship with the CL/F according to Xpose but its influence was not significative when it was tested in the final model, in agreement with the few published results (Lubomirov 2010, Hartkoorn 2010). However, the trend observed in this study could reflect the impaired activity of the SLCO influx transporters as has been also reported to rs4149056 in SLCO1B1.

The DRD3 rs1486012 and 5HT2C_CYS23SER are located in genes encoding for dopamine and serotonin receptors, respectively, which are situated in a diversity of places within the body and thus could be involved in the ADME process of the studied drug though their neurotransmitter-mediated action (Peschl 1978, Varga 2011). Surprisingly, important statistical significances these SNPs on the relationships with the PK parameters of LPV/r in the preliminary analysis were found. According to NONMEM results, the presence of rs1486012 A in homozygosis showed a slightly reduction in drug elimination. In this sense, because it is known that the dopamine modulates the hepatic blood flow (Peschl 1978), maybe the rare alleles could affect the affinity of receptors DRD3 for the dopamine, and a lower activity of them could decrease the hepatic blood flow and hence the degree of LPV/r metabolism. Moreover, the dopamine action seems to be negatively regulated by a serotonin-dependent system (Le Marquand 1994). In this sense, the presence of the allelic variant of 5HT2C_CYS23SER led to an increased CL/F by a factor of 1.12 and 1.04, in heterozygosis and homozygosis, respectively, which could be related with the

mechanism mentioned above. In this case, the influence of the SNP on CL/F was also lower than the 20% required being clinically relevant. Therefore, none of these two SNPs could be included in the final model because they did not fulfill the required diagnostic criteria (Table 3, models 7 and 8). To date no information about the influence of these SNPs in PK behavior of drugs is available in the literature. Therefore it should be advisable to research deeply the PK influence of these SNPs.

It seems probable a CL/F increased in patients with the polymorphism in CYP3A5 rs28371764, and into a lower degree with MRP-2 rs8187707 and SLCO1B1 rs11045819 and rs4149032 genetic variants (these three last with changes < 20% were excluded of the model); while the reduction in this PK parameter could be attributed to the presence of CYP3A4_1461ins and CYP3A rs6945984. However, these results could be inconclusive because of the low frequency of some of the SNPs studied in this population and the reduced number of patients genotyped (109 individuals) (Table 2). Therefore further genetic studies are needed to confirm the SNPs that are clinically relevant on the ADME process of LPV/r.

According to the PK/PG model proposed, the estimated average CL/F value in a standard patient with a wild-type CYP3A5 rs28371764, CYP3A rs6945984 and CYP3A4_1461ins genotype was 3.93 L/h, which falls within the lower limit of the range of mean values outlined in the literature (4.1 to 11 L /h) (Moltó 2008, Crommentuyn 2005, Lubomirov 2010, Crommentuyn 2004). Although the value of IIV of the LPV/r estimated PK parameter showed high remaining intersubject variability that could not be explained by the covariates included in the final model (17.72%), the magnitude was lower than in other PK studies (24-37%) (Aspiroz 2011, Moltó 2008,

Crommentuyn 2004, Bouillon-Pichault 2009). Plots in Figure 3 suggested an important improvement in the VPC and NPC when the final model was compared to the base model, showing the quality of the fit and the adequate description of the measured LPV/r concentrations by the population PK/PG model proposed. The accuracy of the final model was also confirmed by the evaluation analysis using NPDE.

Most of the patients included in this study showed a C^{ss}_{min} value significantly higher than the recommended in the literature (Masqueliar 2002, Gutiérrez 2003, Van der Leur 2006) during the treatment with LPV/r standard dose (400/100 mg bid) (Table 1). Moreover, their mean lipid levels were within or over the limit of normal. In this sense, LPV/r treatment has been related with lipid disorders and metabolic syndrome (Carr 1998, Bozzette 2003, Gutiérrez 2003, González de Requena 2004).

According to the LPV/r population PK/PG model proposed in HIV-infected individuals, Table 5 shows the "a priori" LPV/r dose recommended for different situations. It can be seen that a dose of 300/75 mg bid seems to be adequate for a standard patient without the presence of the relevant genetic variants while the standard dose (400/100 mg bid) would be recommended only for patients with the rs28371764 C>T polymorphism. However, the manufacturer maintains the recommended dose of 400/100 mg bid for all the adult patients since the authorization of the drug, leading to C^{ss}_{min} of 7.58 mg/L or greater if some SNPs are present, which could be responsible for LPV/r-related toxicity (Gutiérrez 2003, González de Requena 2004).

Finally, the implementation of the PK/PG model proposed in Bayesian algorithm could be useful to individualize the LPV/r dose in VIH-infected patients based on data obtained from the TDM.

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Table 1. Demographic, treatment and clinical characteristics of the patients included in the study

CHARACTERISTICS	VALUE Mean ± SD (range) or N (%)
Demographic	
N° of patients	109
Gender (male)	79 (72.47)
Age (years)	41.51 ± 9.46 (19-75)
Total body weight (kg)	67 ± 14.58 (37-125)
Height (cm)	168.8 ± 8.6 (150-189)
Body mass index (kg/m^2)	23.25 ± 4.07 (15.06-38.75)
N° of LPV/r concentrations	693
N° of concentrations per patient	6.36 ± 3.2 (1-13)
LPV/r plasma concentration (mg/L)	8.3 ± 3.15(1.06-23.6)
RTV plasma concentration (mg/L)	1.05 ± 0.55 (0.19-5.51)
Treatment	
Meltrex® formulation*	553 (79.8)
Soft capsules formulation*	121 (17.46)
Liquid formulation*	19 (2.74)
LPV/r dailly dose/12 h (mg/12 h)*	394.56 ± 31.68 (200-480)
Biochemical	
TB (mg/dL)*	0.86 ± 0.58 (0.1-5.3)
TG(mg/dL)*	220.10 ± 157.93 (44-1427)
TC (mg/dL)*	192.04 ± 46.92 (73-333)
cLDL (mg/dL)*	102.45 ± 37.36 (24-218)
cHDL (mg/dL)*	46.80 ± 16.06 (15-126)
cLDL/cHDL*	2.32 ± 1.01 (0.2-6.6)
CD_{4+} [x $10^6/\mu L$]*	422.81 ± 251.63 (24-1459)
Plasma viral load detectable*	451 (18.53)
Concomitant treatment	
TFV*	505 (72.87)
Concomitant patologies	
HCV*	327 (47.19)

*Data referenced to the number of LPV/r concentrations analyzed.

Data concerning demographic and treatment characteristics, biochemical parameters related to liver function (total bilirubin (TB)), lipid profile (triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (cLDL), high-density lipoprotein cholesterol (cHDL), low/high-density lipoprotein cholesterol ratio (cLDL/cHDL)), clinical evolution ($CD_{4+,}$ plasma viral load), concomitant treatment (tenofovir (TFV)) and concomitant pathologies (hepatitis C virus (HCV)).

Table 2. Genotype data of analyzed SNPs

GENE	SNP	GENOTYPE				QUALITY CONTROL		
		Total (N)	Wild-type (%)	Heterozygous (%)	Homozygous (%)	HWE (prob)	Genotype Rate (%)	MAF
ABCA1	rs4149313 A>G	109	70.64	25.69	3.67	0.48	100	0.165
ABCB1	rs2032582 G>T	107	53.27	38.32	8.41	0.67	100	0.276
ABCB1	rs1045642 C>T	109	33.03	44.95	22.02	0.35	100	0.445
ABCB1	rs1128503 C>T	109	46.79	42.2	11.01	0.74	100	0.321
ABCB1	rs2229109 G>A	109	98.17	1.83	0	0.92	100	0.009
ABCB1	rs2235046 G>A	108	45.37	43.52	11.11	0.89	99.08	0.329
ABCB1	rs3789243 T>C	109	39.45	44.95	15.6	0.63	100	0.381
ABCB1	rs9282564 A>G	108	90.74	8.33	0.93	0.15	99.08	0.051
ABCC1	rs246221 T>C	95	53.68	36.84	9.47	0.41	87.16	0.279
ABCC1	rs35587 T>C	109	44.04	45.87	10.09	0.70	100	0.330
ABCC1	rs45560437 G>A	109	84.4	15.6	0	0.38	100	0.078
ABCC2	ABCC2_259 G>T	108	96.3	3.7	0	0.84	99.08	0.019
ABCC2	rs17222723 T>A	108	88.89	11.11	0	0.54	99.08	0.056
ABCC2	rs2273697 G>A	107	63.55	30.84	5.61	0.46	98.17	0.210
ABCC2	rs3740066 G>A	98	38.78	44.9	16.33	0.59	89.91	0.388
ABCC2	rs7080681 G>A	109	100	0	0		100	0.000
ABCC2	rs717620 G>A	108	62.04	30.56	7.41	0.18	99.08	0.227
ABCC2	rs8187707 C>T	109	88.07	11.93	0	0.51	100	0.060
ABCC4	rs11568658 G>T	108	96.3	3.7	0	0.84	99.08	0.019
ABCC4	rs11568695 G>A	109	98.17	1.83	0	0.92	100	0.009
ABCC4	rs12875235 T>G	107	73.83	25.23	0.93	0.43	98.17	0.136
ABCC4	rs1557070 C>T	109	98.17	1.83	0	0.92	100	0.009
ABCC4	rs1751034 T>C	109	60.55	37.61	1.83	0.12	100	0.206
ABCC4	rs2274405 C>T	109	34.86	50.46	14.68	0.59	100	0.399
ABCC4	rs2274406 C>T	108	33.33	50	16.67	0.77	99.08	0.417
ABCC4	rs2274407 C>A	109	85.32	14.68	0	0.41	100	0.073
ABCC4	rs3742106 A>C	109	34.86	51.38	13.76	0.43	100	0.394
ABCC4	rs45616431 T>C	108	98.15	1.85	0	0.92	99.08	0.009
ABCC4	rs899494 G>A	107	67.29	24.3	8.41	0.01	98.17	0.206
ABCG2	M376CT	108	100	0	0		99.08	0.000
ABCG2	rs2231137 G>A	108	87.96	12.04	0	0.51	99.08	0.060
ABCG2	rs2231142 C>A	108	86.11	13.89	0	0.44	99.08	0.069
CETP	rs1800775 C>A	109	27.52	47.71	24.77	0.64	100	0.486
CYP1A2	rs762551 A>C	105	43.81	44.76	11.43	1.00	96.33	0.338
CYP1A2	CYP1A _3534GA	108	100	0	0		99.08	0.000
CYP1A2	rs72547513 C>A	109	100	0	0		100	0.000

CYP1A2	CYP1A2_634AT	109	100	0	0		100	0.000
CYP1A2	rs2069526 T>G	108	98.15	1.85	0	0.92	99.08	0.009
CYP2B6	rs3211371 C>T	109	78.9	21.1	0	0.22	100	0.106
CYP2B6	rs3745274 G>T	109	59.63	35.78	4.59	0.78	100	0.225
CYP2B6	rs8192709 C>T	109	88.99	11.01	0	0.54	100	0.055
CYP2C19	rs12248560 C>T	109	59.63	35.78	4.59	0.78	100	0.225
CYP2C19	rs41291556 T>C	109	99.08	0.92	0	0.96	100	0.005
CYP2C19	rs4244285 G>A	109	81.65	16.51	1.83	0.35	100	0.101
CYP2C19	rs4986893 G>A	109	100	0	0		100	0.000
CYP2C8	rs10509681 T>C	109	68.81	29.36	1.83	0.50	100	0.165
CYP2C8	rs11572080 G>A	109	68.81	29.36	1.83	0.50	100	0.165
CYP2C8	rs11572103 A>T	104	94.23	5.77	0	0.76	95.41	0.029
CYP2C9	rs1057909	109	100	0	0		100	0.000
CYP2C9	rs1057910 A>C	106	100	0	0		97.25	0.000
CYP2C9	rs1057911 A>T	109	84.4	15.6	0	0.38	100	0.078
CYP2C9	rs2256871 T>C	109	99.08	0.92	0	0.96	100	0.005
CYP2C9	rs28371685 C>T	109	100	0	0		100	0.000
CYP2C9	rs9332130 A>G	109	100	0	0		100	0.000
CYP2C9	rs9332239 C>T	109	100	0	0		100	0.000
CYP3A	rs6945984 T>C	102	81.37	17.65	0.98	0.98	93.58	0.098
CYP3A4	1461_1462insA	109	99.08	0.92	0	0.96	100	0.005
CYP3A4	rs4987161	109	100	0	0		100	0.000
CYP3A4	rs12721629	109	100	0	0		100	0.000
CYP3A4	P416L	109	100	0	0		100	0.000
CYP3A4	R130Q	109	100	0	0		100	0.000
CYP3A4	T185SmodC	109	100	0	0		100	0.000
CYP3A4	T185SmodT	109	100	0	0		100	0.000
CYP3A4	T363M	109	100	0	0		100	0.000
CYP3A4	rs2740574 A>G	109	88.99	9.17	1.83	0.01	100	0.064
CYP3A4	rs28371759 T>G	109	100	0	0		100	0.000
CYP3A5	rs10264272 C>T	109	100	0	0		100	0.000
CYP3A5	rs28365085 T>C	109	100	0	0		100	0.000
CYP3A5	rs28371764 C>T	109	90.83	9.17	0	0.62	100	0.046
CYP3A5	rs41279854 A>G	109	100	0	0		100	0.000
CYP3A5	rs4646453 G>T	109	96.33	3.67	0	0.85	100	0.018
CYP3A7	rs2257401 G>C	108	88.89	11.11	0	0.54	99.08	0.056
DRD2	141C_del	108	79.63	17.59	2.78	0.14	99.08	0.120
DRD2	Ser311Cys C>G	109	97.25	2.75	0	0.88	100	0.014
DRD3	rs1486012 T>A	109	36.7	44.95	18.35	0.47	100	0.408
DRD3	rs2134655 G>A	109	48.62	44.04	7.34	0.52	100	0.294
DRD3	rs963468 G>A	109	44.95	40.37	14.68	0.25	100	0.349

DYPYP	DPYP_2A G>A	109	100	0	0		100	0.000
NR1I2_PXR	rs1523130 G>A	109	36.7	42.2	21.1	0.16	100	0.422
NR1I2_PXR	rs2472677 T>C	107	32.71	49.53	17.76	0.89	98.17	0.425
NR1I2_PXR	rs763645 C>T	109	23.85	58.72	17.43	0.06	100	0.468
ORM1	ORM1_113 A>G	105	100	0	0		96.33	0.000
ORM1	rs10982151 T>G	108	66.67	31.48	1.85	0.37	99.08	0.176
ORM1	rs10982158 A>T	105	69.52	30.48	0	0.07	96.33	0.152
SLC22A6	rs4149170 G>A	105	80	18.1	1.9	0.46	96.33	0.110
SLCO1A2	rs10841795 A>G	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs10841798 T>G	109	88.07	11.93	0	0.51	100	0.060
SLCO1A2	rs10841803 G>A	109	69.72	28.44	1.83	0.57	100	0.161
SLCO1A2	rs11045918 C>A	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs11045923 C>G	108	35.19	52.78	12.04	0.23	99.08	0.384
SLCO1A2	rs11568563 A>C	109	88.99	11.01	0	0.54	100	0.055
SLCO1A2	rs11837182 C>T	109	81.65	17.43	0.92	0.99	100	0.096
SLCO1A2	rs16923597 A>G	109	66.97	28.44	4.59	0.47	100	0.188
SLCO1A2	rs4149006 C>A	108	66.67	30.56	2.78	0.73	99.08	0.181
SLCO1A2	rs4149008 C>T	109	67.89	27.52	4.59	0.40	100	0.183
SLCO1A2	rs4337089 C>T	109	67.89	29.36	2.75	0.84	100	0.174
SLCO1A2	rs5484 C>T	109	72.48	23.85	3.67	0.33	100	0.156
SLCO1A2	rs6487216 T>C	109	39.45	48.62	11.93	0.59	100	0.362
SLCO1A2	rs722994 G>A	107	74.77	20.56	4.67	0.05	98.17	0.150
SLCO1A2	rs7967770 A>C	107	78.5	20.56	0.93	0.74	98.17	0.112
SLCO1B1	rs11045819 C>A	109	71.56	24.77	3.67	0.40	100	0.161
SLCO1B1	rs17328763 T>C	108	60.19	33.33	6.48	0.51	99.08	0.231
SLCO1B1	rs2306283 T>C	109	29.36	49.54	21.1	0.98	100	0.459
SLCO1B1	rs4149015 G>A	109	83.49	14.68	1.83	0.21	100	0.092
SLCO1B1	rs4149032 C>T	109	35.78	45.87	18.35	0.57	100	0.413
SLCO1B1	rs4149056 T>C	109	73.39	22.94	3.67	0.26	100	0.151
SLCO1B3	rs4149117 G>T	109	79.82	16.51	3.67	0.03	100	0.119
SLCO2B1	rs1077858 A>G	109	42.2	44.95	12.84	0.87	100	0.353
TPMT	rs1142345 A>G	109	95.41	4.59	0	0.81	100	0.023
TPMT	rs1800460 G>A	108	96.3	3.7	0	0.84	99.08	0.019
5HT2A	5HT2A _102TC	109	27.52	54.13	18.35	0.83	100	0.454
5HT2A	HIS452TYR	109	79.82	19.27	0.92	0.00	100	0.106
5HT2C	CYS23SER	109	77.06	11.01	11.93	0.48	100	0.174

HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency.

Table 3. Representative PK/PG population models tested for CL/F

Model no.	Model description	Covariates	OFV	Model no. used for comparison	DOFV	$CV_{CL/F}$ (%)	σ (%)
1	$CL/F = 3.96$	Basic model	1699.656			26.65	20.47
2*	$CL/F = (0.220 \cdot BMI + 0.0112 \cdot cHDL) \cdot 0.715^{RTC}$	BMI, cHDL, RTC	1421.414	1	278.242	20.10	17.23
3	$CL/F = (0.209 \cdot BMI + 0.00769 \cdot cHDL) \cdot 0.721^{RTC} \cdot 1.27^{rs28371764\ [C/T]}$	BMI, cHDL, RTC, rs28371764C>T	1404.741	2	16.673	19.42	17.15
4	$CL/F = (0.197 \cdot BMI + 0.00985 \cdot cHDL) \cdot 0.721^{RTC} \cdot 1.22^{rs28371764\ [C/T]} \cdot 1.12^{rs4149032\ [C/T]} \cdot 1.10^{rs4149032\ [T/T]}$	BMI, cHDL, RTC, rs28371764C>T, rs4149032C>T	1393.359	3	11.382	18.55	17.18
5	$CL/F = (0.210 \cdot BMI + 0.0114 \cdot cHDL) \cdot 0.717^{RTC} \cdot 1.19^{rs28371764\ [C/T]} \cdot 1.13^{rs8187707\ [C/T]}$	BMI, cHDL, RTC, rs28371764C>T, rs8187707C>T	1399.313	3	5.428	19.14	17.18
6	$CL/F = (0.212 \cdot BMI + 0.0118 \cdot cHDL) \cdot 0.719^{RTC} \cdot 1.25^{rs28371764\ [C/T]} \cdot 0.974^{rs6945984\ [T/C]} \cdot 0.526^{rs6945984\ [C/C]}$	BMI, cHDL, RTC, rs28371764C>T, rs6945984T>C	1397.935	3	6.806	18.84	17.15
7	$CL/F = (0.211 \cdot BMI + 0.0116 \cdot cHDL) \cdot 0.720^{RTC} \cdot 1.26^{rs28371764\ [C/T]} \cdot 0.528^{rs6945984\ [C/C]}$	BMI, cHDL, RTC, rs28371764C>T, rs6945984T>C	1398.230	3	6.511	18.92	17.15
8	$CL/F = (0.215 \cdot BMI + 0.012 \cdot cHDL) \cdot 0.721^{RTC} \cdot 1.24^{rs28371764\ [C/T]} \cdot 0.969^{rs1486012\ [T/A]} \cdot 0.944^{rs1486012\ [A/A]}$	BMI, cHDL, RTC, rs28371764C>T, rs1486012T>A	1403.389	3	1.352	19.29	17.18
9	$CL/F = (0.207 \cdot BMI + 0.0107 \cdot cHDL) \cdot 0.722^{RTC} \cdot 1.24^{rs28371764\ [C/T]} \cdot 1.12^{5HT2C\_23CYS/SER} \cdot 1.04^{5HT2C\_23SER/SER}$	BMI, cHDL, RTC, rs28371764C>T, 5HT2C_CYS23SER	1400.397	3	4.344	19.29	17.12
10	$CL/F = (0.204 \cdot BMI + 0.011 \cdot cHDL) \cdot 0.721^{RTC} \cdot 1.26^{rs28371764\ [C/T]} \cdot 1.11^{rs11045819\ [C/A]} \cdot 1.03^{rs11045819\ [A/A]}$	BMI, cHDL, RTC, rs28371764C>T, rs11045819C>A	1397.895	3	6.846	19.00	17.15
11	$CL/F = (0.210 \cdot BMI + 0.0121 \cdot cHDL) \cdot 0.721^{RTC} \cdot 1.26^{rs28371764\ [C/T]} \cdot 0.305^{CYP3A4\ [1461insA/del]}$	BMI, cHDL, RTC, rs28371764C>T, CYP3A4_1461ins	1389.285	3	15.456	18.14	17.18
12	$CL/F = (0.216 \cdot BMI + 0.0125 \cdot cHDL) \cdot 0.713^{RTC} \cdot 1.26^{rs28371764\ [C/T]} \cdot 0.528^{rs6945984\ [C/C]} \cdot 0.302^{CYP3A4\ [1461insA/del]}$	BMI, cHDL, RTC, rs28371764C>T, rs6945984T>C, CYP3A4_1461ins	1375.265	11	14.020	17.72	17.12

*Intermediate model.

Table 4. Parameter estimates and estimated standard errors (SE) for LPV/r final PK/PG population model[a]

Parameter	Estimate	SE (%)*
θ_1 (L/h)	0.216	0.00915 (4.23)
θ_2 (L)	130	-
θ_3 (h^{-1})	1.90	-
θ_4 (h)	1.78	-
θ_5	0.0125	0.00409 (32.72)
θ_6	0.713	0.0173 (2.43)
θ_7	1.26	0.125 (9.92)
θ_8	0.528	0.0104 (1.97)
θ_9	0.302	0.00687 (2.27)
$CV_{CL/F}$ (%)	17.72	0.00535 (17.04)
σ (%)	17.12	0.00224 (7.65)

[a]Final model: $CL/F_{LPV} = (\theta_1 \cdot BMI + \theta_5 \cdot cHDL) \cdot \theta_6^{RTC} \cdot \theta_7^{rs28371764\ [C/T]} \cdot \theta_8^{rs6945984\ [C/C]} \cdot \theta_9^{CYP3A4\ [1461insA/del]}$; $V/F = \theta_2$; $Ka = \theta_3$; $ALAG = \theta_4$.

*The SE as % was calculated from the mean value of the parameter estimate.

Table 5. LPV/r dose recommendations for the different genotypes according to the final population PK/PG model. Calculations were made for standard patients with mean values of the studied population (BMI = 23.25 kg/m^2, cHDL = 46.80 mg/dL, RTC = 1.05 mg/L)

SNP Genotype	Frequency (%)*	CL/F (L/h)	C^{ss}_{min} (mg/L) for 400/100mg bid	Dose recommendation	C^{ss}_{min} (mg/L) estimated for the recommended dose
Wild-type	88.9	3.93	7.58	300/75 mg bid	5.68
rs28371764 C/T	9.17	4.95	5.73	Standard dose	5.73
rs6945984 C/C	0.98	2.08	15.29	300/75 mg qd	5.19
CYP3A4_1461insA/del	0.92	1.19	28.19	200/50 mg qd	6.67
				160/40 mg qd	5.34

*Among the studied population.
Bid: twice daily; qd: once a day.

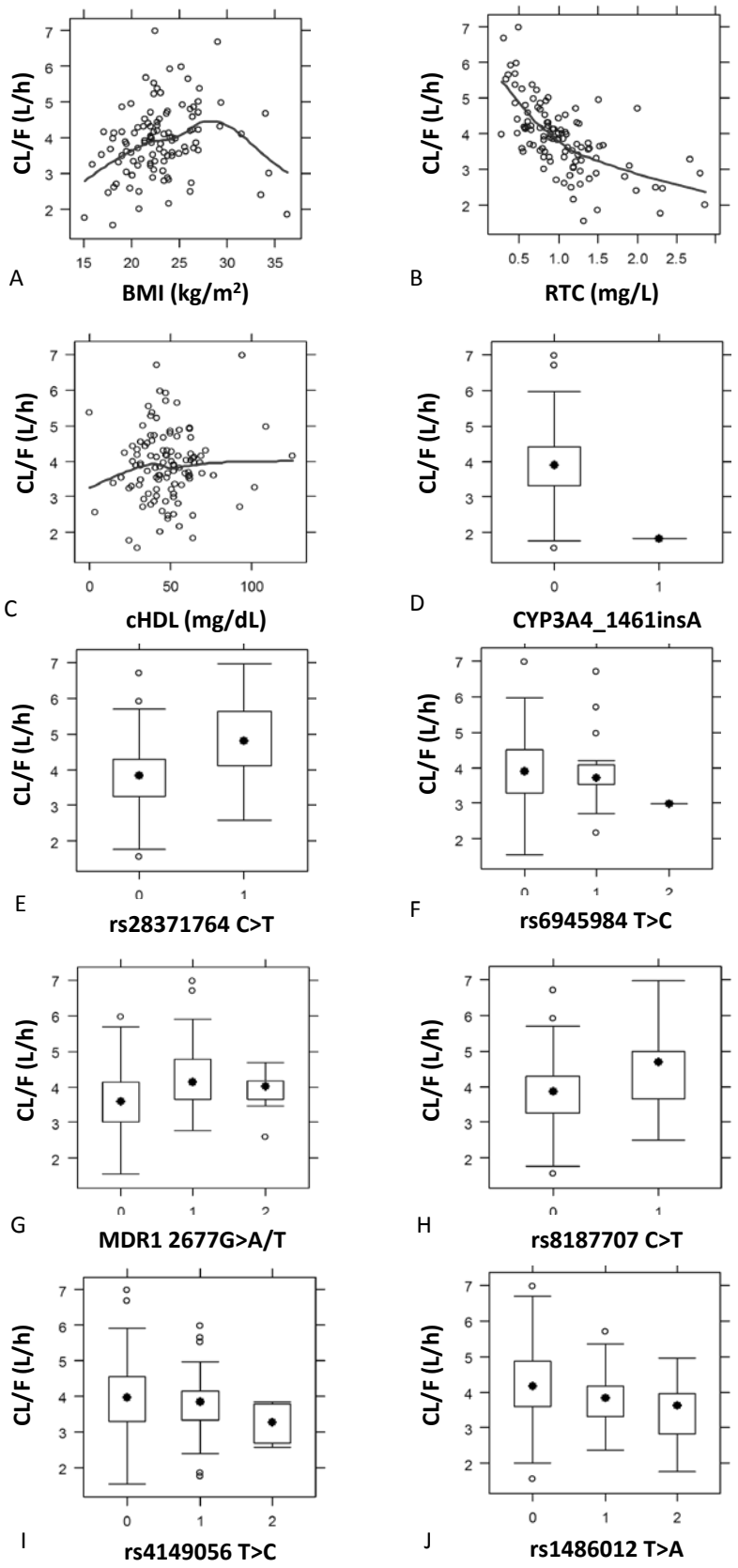

Figure 1. Relationships between post hoc Bayesian LPV/r oral clearance (CL/F) and (A) BMI, (B) RTC, (C) cHDL and (D to J) the wild-type, heterozygous or homozygous genotype (0, 1 or 2, respectively) for the SNPs in CYP3A4_1461ins, rs28371764, rs6945984, MDR1 2677, rs8187707, rs4149056 and rs1486012.

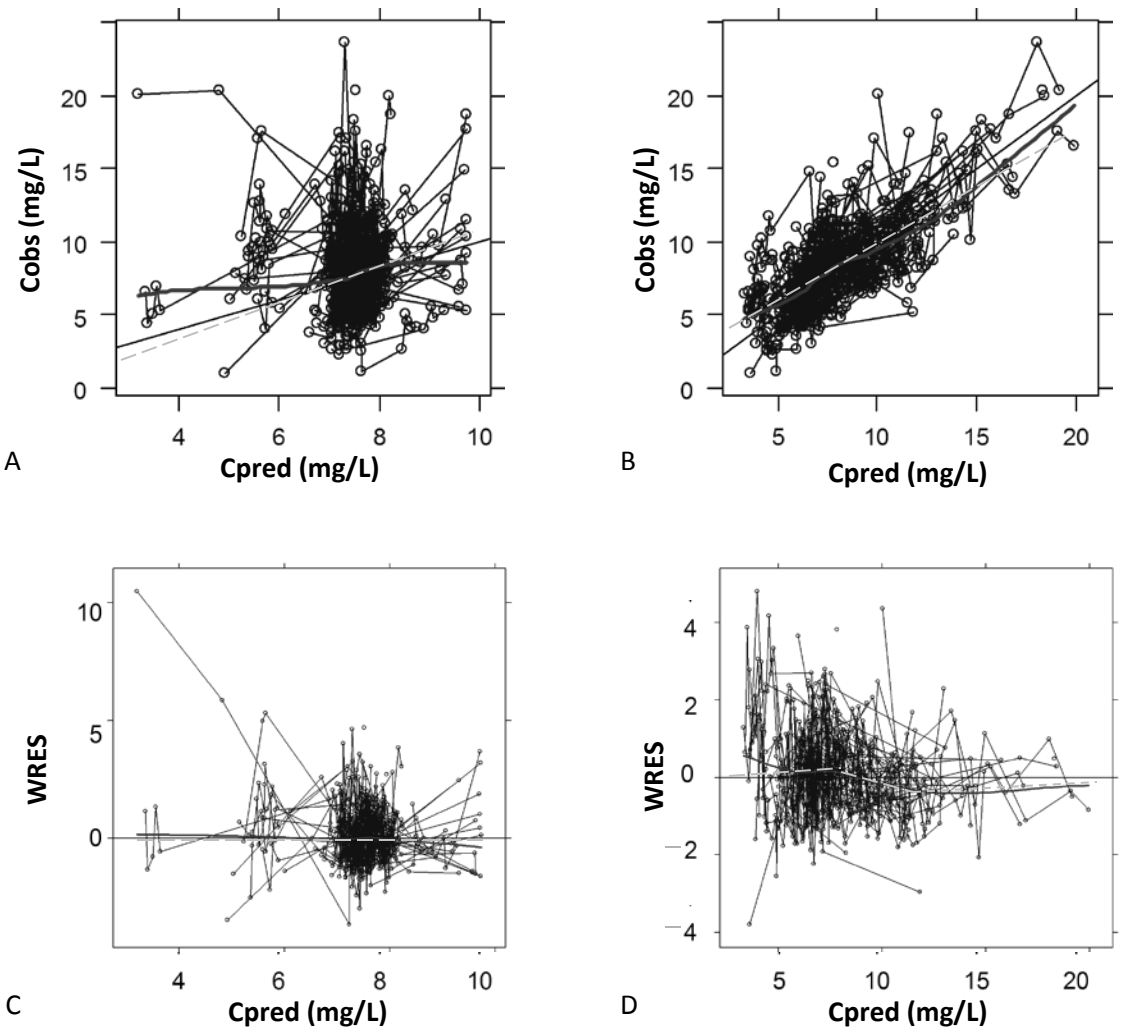

Figure 2. Observed LPV/r plasma concentrations (Cobs) versus predicted population concentrations (Cpred) from (A) the basic and (B) the final models; and weight residuals (WRES) versus population Cpred from (C) the basic and (D) the final models.

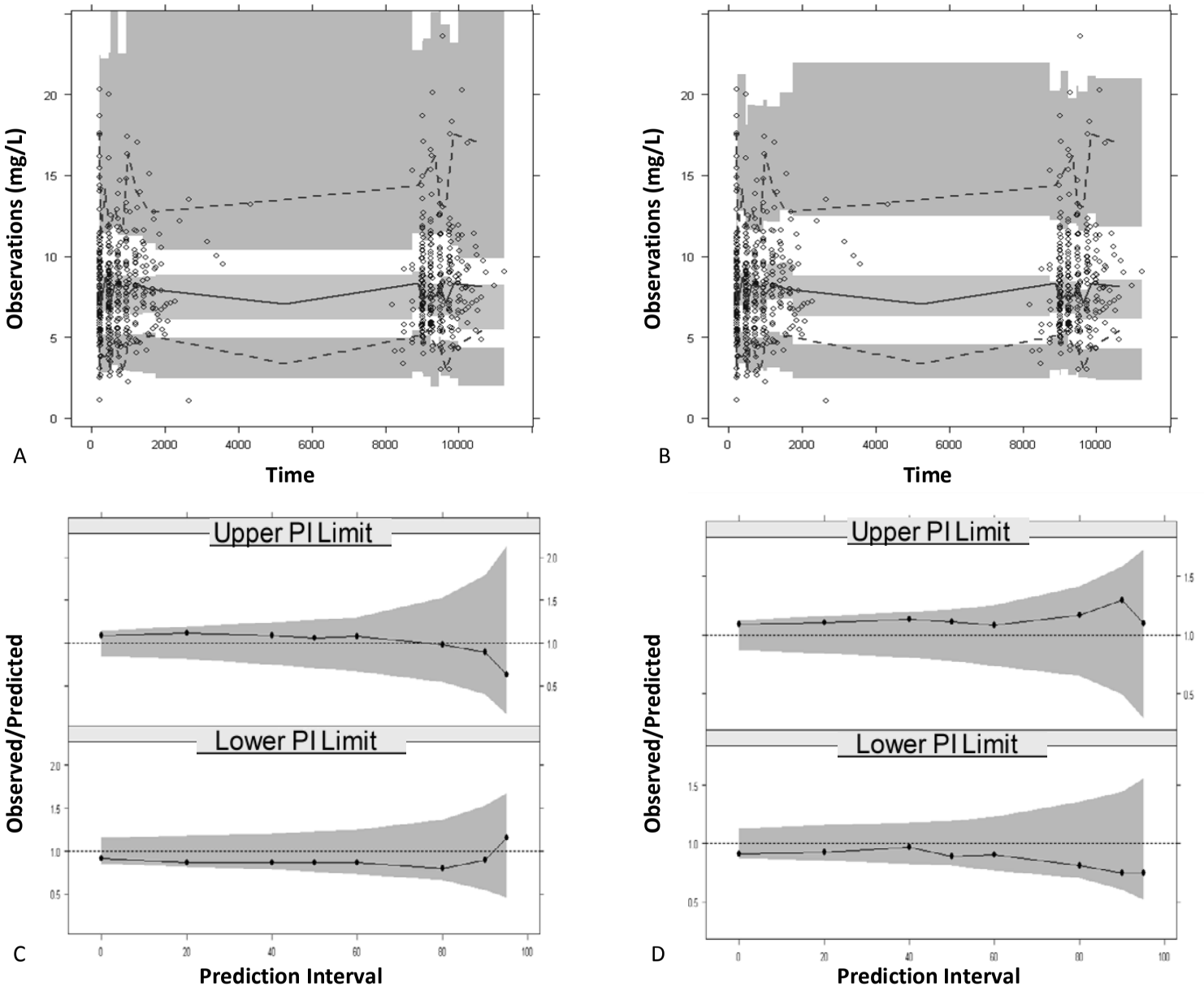

Figure 3. Visual predictive checks for (A) basic and (B) final models. Dotted lines: 10th and 90th percentiles; continuous line: 50th percentile; and plots of the results from the numerical predictive check for (C) basic and (D) final models.

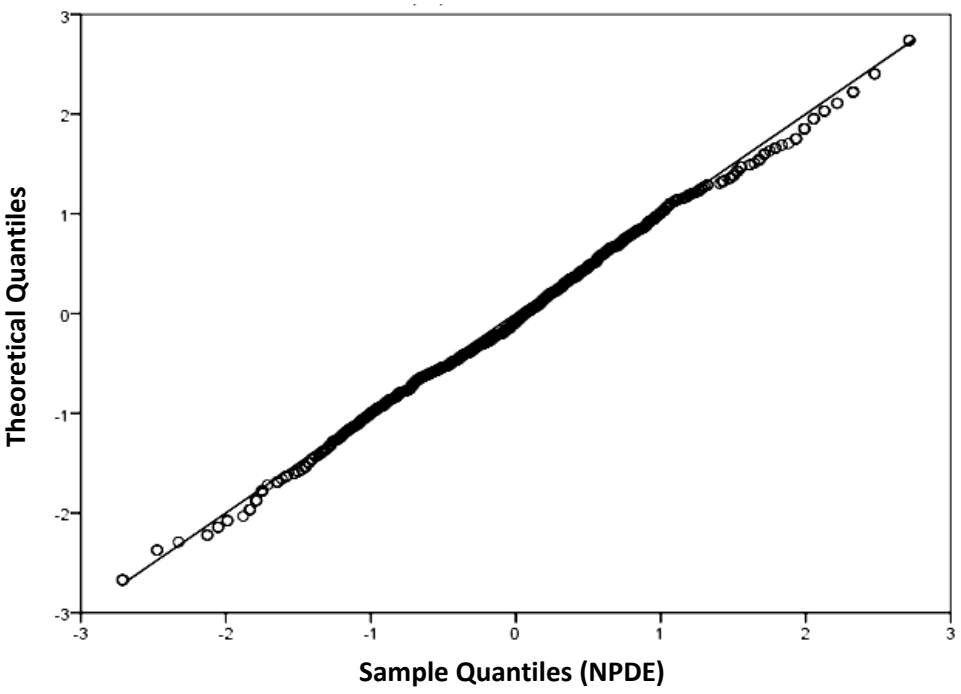

Figure 4. Quantile-quantile plots of the LPV/r NPDE distribution.

IV. CONCLUSIONES

✓ La información bibliográfica revisada pone de manifiesto la importancia de lopinavir/ritonavir (LPV/r) como componente del tratamiento antirretroviral, debido a la adecuada eficacia y seguridad demostrada en la práctica clínica. Los resultados publicados muestran una elevada variabilidad interindividual en su comportamiento farmacocinético (PK), la respuesta inmunovirológica y la presencia de efectos adversos tras la administración de la dosis estándar de 400/100 mg cada 12 horas, lo que sugiere la búsqueda de nuevas estrategias dirigidas a la optimización del tratamiento con este fármaco.

✓ Se confirma el efecto potenciador o *"boosting"* de ritonavir (RTV) sobre lopinavir (LPV), y se demuestra una relación directa entre el área bajo la curva de RTV y su concentración al final del intervalo posológico, lo que permite utilizar este último parámetro como predictor del efecto potenciador. Esta aportación resulta de gran utilidad debido a la mayor facilidad de obtener una única concentración al final del intervalo, frente a la caracterización de perfiles cinéticos completos.

✓ Se ha desarrollado y validado un modelo PK poblacional preliminar para LPV en pacientes VIH+ en el que se han identificado el índice de masa corporal, la concentración mínima de RTV y la administración concomitante de efavirenz y atazanavir como covariables que afectan de forma significativa su comportamiento PK. En el mismo grupo de pacientes se ha desarrollado y validado un modelo PK poblacional para en el que se han identificado como covaribles significativas la administración concomitante de efavirenz, saquinavir y atazanavir.

✓ Los resultados obtenidos sobre la influencia de los 290 SNPs estudiados en 109 pacientes VIH+ sobre la farmacocinética, la eficacia y la toxicidad de LPV/r a través de un análisis univariante, un análisis longitudinal y una regresión logística, proporcionan

una información preliminar sobre la potencial influencia de algunos de estos polimorfismos sobre el comportamiento del fármaco.

- ✓ El modelo farmacocinético/farmacogenético (PK/PG) poblacional desarrollado y validado para LPV/r, identifica los SNPs CYP3A4_1461ins, CYP3A rs6945984 y CYP3A5 rs28371764 como covariables farmacogenéticas con probable influencia en la cinética de disposición de este fármaco. Se requieren estudios complementarios que confirmen estos resultados debido a la baja representatividad en la población de estudio de algunas de las covariables genéticas analizadas.
- ✓ Se ha implementado el modelo PK/PG poblacional de LPV/r propuesto en el programa de farmacocinética clínica PKS® lo que permite estimar con fiabilidad los parámetros farmacocinéticos de los pacientes monitorizados utilizando métodos Bayesianos y de acuerdo con ellos y con el control clínico de la enfermedad, ajustar la posología de forma individualizada.