Single Nucleotide Polymorphisms in *UGT1A1* Associate with Atazanavir-related Nephrolithiasis

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Abstract

Background: Ritonavir-boosted atazanavir (ATV/r) is a widely used antiretroviral drug though it can cause nephrolithiasis. The aim of this study was to determine the association between polymorphisms in genes encoding proteins that take part in metabolizing process of ATV and drug transporters, and ATV/r-associated nephrolithiasis in HIV-1-infected patients treated with ATV/r.

Methods: The 19 single nucleotide polymorphisms (SNP) in the *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1*, and *CYP3A5* genes were examined in case patients with ATV/r-induced nephrolithiasis (n=31) and controls (n=47). Case patients were those with a clinical diagnosis of nephrolithiasis while on ATV/r, based on new onset of acute flank pain plus one of the following: 1) new-onset hematuria, 2) documented presence of stones by either abdominal ultrasonography or computed tomography, 3) confirmed stone passage. Control patients were consecutively enrolled among those with >2 years of ATV/r experience in whom nephrolithiasis did not occur. Genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols. Associations between alleles and ATV/r-associated nephrolithiasis were tested by univariate and multivariate logistic regression analyses.

Results:
None of patient characteristics variables (sex, age, body weight, body mass index, CD4 count, renal function, serum uric acid, co-administration of tenofovir, history of nephrolithiasis and indinavir use) was associated with ATV/r-associated nephrolithiasis. Multivariate analyses adjusted with sex and age showed a significant association between ATV/r-associated nephrolithiasis and any T allele at position c.211 (rs10929303, adjusted OR=3.660; 95%CI, 1.163-11.52; p=0.027), any G allele at position 339 (rs1042640, adjusted OR=5.704; 95%CI, 1.592-20.44; p=0.008), and any G allele at position 440 (rs8330, adjusted OR=5.704; 95%CI, 1.592-20.44; p=0.008) of UGT1A1.

Conclusions: This is the first study to identify the association between SNPs in *UGT1A1* and atazanavir-associated nephrolithiasis. Atazanavir-treated patients with these SNPs might be at higher risk of atazanavir-associated nephrolithiasis than those without.

Background

- ✓ The incidence of nephrolithiasis is substantially higher in patients on ritonavir-boosted atazanavir (ATV/r)-containing antiretroviral therapy (ART) than patients on other PI- or efavirenz-containing ART.[1),2)]
- ✓ The development of renal stones, even a single episode, is a risk factor for significant decrement in renal function.[3)]
- ✓ ATV is mainly metabolized by cytochrome P450 (CYP3A), including *CYP3A4* and *CYP3A5*,[4)] and UDP-glucuronosyltransferase 1A1 (*UGT1A1*), is also involved in the metabolism of ATV.[5)]
- ✓ P-glycoprotein (P-gp) is a membrane protein which regulates ATV intestinal absorption, and thus affects exposure to ATV.[6)] P-gp is encoded by the *ABCB1* gene.
- ✓ The organic anion-transporting polypeptide 1B1 (OATP1B1), encoded by the gene *SLCO1B1*, is another protein involved in influx transportation of ATV.[7)]
- ✓ Single nucleotide polymorphisms (SNP) in the *NR1I2* gene, which encodes the nuclear receptor pregnane X receptor (PXR), regulate the expression of *CYP3A4* and *ABCB1*, and also influence ATV concentration.[8)]
- ✓ The present study was designed to elucidate the association between polymorphisms in genes encoding abovementioned proteins and ATV-induced nephrolithiasis.

Methods

A single-center, case-control study to elucidate the association between SNPs in genes encoding metabolism and transporter proteins for ATV and ATV-induced nephrolithiasis.

***Study entry criteria*:** HIV-infected Japanese patients aged >17 years, who commenced treatment with ATV/r-containing ART between Jan 2004 and Jun 2012, regardless of being treatment-naïve or experienced. Enrollment took place from September 2012 to March 2013.

***Exclusion criteria*:** 1) commenced ATV/r-containing ART at other facilities, 2) prescribed unboosted ATV.

***Case patients*:** To whom nephrolithiasis occurred while on ATV/r containing ART. Nephrolithiasis was defined as following: a clinical diagnosis by the attending physician based on new onset of acute flank pain plus one of the following: 1) new-onset hematuria confirmed by urine dipstick test, 2) documented presence of stones or radiological findings suggestive of renal stones, by either abdominal ultrasonography or computed tomography, 3) stone passage confirmed by either the patient or attending physician.

***Control patients*:** consecutively enrolled among those with >2 years of ATV/r experience in whom nephrolithiasis did not occur.

Measurements

We used the data on or closest to and preceding the day of starting ATV/r-containing ART by no more than 180 days, except for total bilirubin (T-Bil), which was collected at 2 years after initiation of ATV/r.

Statistical analysis

19 SNPs in the *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1*, *CYP3A5* genes were investigated.

Genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols.

Associations between genotypes and nephrolithiasis were tested by univariate and multivariate logistic regression analyses.

Results

Table 1. Patient characteristics

	Renal stones (n=31)	Controls (n=47)	P value
Male ¶	29 (94)	42 (89)	0.70
Age†	39 (35-45)	40 (35-49)	0.40
BMI (kg/m^2)†	22.7 (19.9-24.9)	22.7 (20.4-24.6)	0.58
eGFR(ml/min/1.73m^2)†	85.8 (69.7-97.9)	88.5 (78.4-98.6)	0.81
CD4 count (/μl)†	232 (194-356)	229 (187-290)	0.63
HIV load (log$_{10}$/ml)†	3.96 (2.25-4.79)	4.22 (3.41-4.77)	0.65
Uric acid (mg/dl)†	6.3 (5.5-7.7)	5.9 (4.9-6.7)	0.15
History of renal stone¶	4 (13)	2 (4)	0.21
HCV infection¶	**5 (16)**	**1 (2)**	**0.03**
Treatment-naïve¶	18 (58)	32 (68)	0.47
TDF use¶	9 (29)	17 (36)	0.63
History of indinavir¶	3 (10)	3 (6)	0.68
Serum T-Bil (mg/dl)†	2.3 (1.8-3.4)	1.7 (1.3-1.9)	<0.01

Categorical variables were compared by the χ^2 test or Fisher's exact test.
Continuous variables were compared by the Student's t-test.
¶number (%), †median (interquartile range), eGFR: estimated glomerular filtration rate

Results

Table 2. Genotype frequencies

	Renal stone (n=31)	No stone (n=47)	P value*
ABCB1			
193 A→G, rs3842			
A/A	16 (52)	27 (57)	
A/G	14 (45)	14 (30)	0.20
G/G	1 (3)	6 (13)	
365 T→C, rs3213619			
T/T	28 (90)	37 (79)	
T/C	3 (10)	10 (21)	0.23
C/C	0	0	
1236 T→C, rs1128503	Gly412Gly		
T/T	7 (23)	15 (32)	
T/C	19 (61)	22 (47)	0.45
C/C	5 (16)	10 (21)	
2677T→A/G, rs2032582	A:Ser893Thr G:Ser893Ala		
T/T	5 (16)	5 (11)	
T/A	6 (19)	5 (11)	
G/G	3 (10)	10 (21)	0.44
G/T	10 (32)	15 (32)	
G/A	6 (19)	12 (26)	
A/A	1 (3)	0	
3435 C→T, rs1045642	Ile1145Ile		
C/C	10 (32)	22 (47)	
C/T	16 (52)	21 (45)	0.35
T/T	5 (16)	4 (9)	
NR1I2 (Pregnane X receptor)			
131 A→C, rs1523127			
C/C	1 (3)	6 (13)	
C/A	18 (58)	23 (49)	0.33
A/A	12 (39)	18 (38)	
370 G→A, rs3732359			
G/G	8 (26)	16 (34)	
G/A	19 (61)	21 (45)	0.34
A/A	4 (13)	10 (21)	
522 C→T, rs3732360			
C/C	8 (26)	15 (32)	
C/T	19 (61)	23 (49)	0.43
T/T	4 (13)	9 (19)	
1195 A→C, rs3814057			
A/A	4 (13)	11 (23)	
A/C	19 (61)	22 (47)	0.38
C/C	8 (26)	14 (30)	
1232 T→C, rs3814058			
T/T	4 (13)	10 (21)	
T/C	19 (61)	21 (45)	0.34
C/C	8 (26)	16 (34)	

*by Fisher's exact test

Table 2. Genotype frequencies (cont'd)

	Renal stone (n=31)	No stone (n=47)	P value*
UNR1I2 (Pregnane X receptor)			
44477 T→C, rs1523130			
T/T	1 (3)	6 (13)	
T/C	17 (55)	22 (47)	0.34
C/C	13 (42)	19 (40)	
63396 T→C, rs2472677			
T/T	18 (58)	24 (51)	
T/C	9 (29)	20 (43)	0.37
C/C	4 (13)	3 (6)	
UGT1A1			
211 G→A, rs4148323	Gly71Arg		
G/G	19 (61)	30 (64)	
G/A	11 (36)	13 (28)	0.55
A/A	1 (3)	4 (9)	
c.211 T→C, rs10929303			
T/T	**0**	**0**	
T/C	**11 (35)**	**6 (13)**	**0.03**
C/C	**20 (65)**	**41 (87)**	
339 G→C, rs1042640			
G/G	**0**	**0**	
G/C	**11 (35)**	**4 (9)**	**0.01**
C/C	**20 (65)**	**43 (92)**	
440 G→C, rs8330			
G/G	**1 (3)**	**0**	
G/C	**10 (32)**	**4 (9)**	**0.01**
C/C	**20 (65)**	**43 (92)**	
SLCO1B1			
388 A→G, rs2306283	Asn130Asp		
A/A	14 (45)	24 (51)	
A/G	14 (45)	20 (43)	0.74
G/G	3 (10)	3 (6)	
521 T→C, rs4149056	Val174Ala		
T/T	20 (65)	31 (66)	
T/C	11 (36)	13 (28)	0.31
C/C	0	3 (6)	
CYP3A5			
14 T→C, rs15524			
T/T	16 (52)	25 (53)	
T/C	14 (45)	17 (36)	0.42
C/C	1 (3)	5 (11)	

*by Fisher's exact test

Table 3. Univariate analysis to estimate a risk of various factors in ATV-induced nephrolithiasis.

	OR	95% CI	P value
Male sex	1.726	0.313-9.513	0.531
Age per 1 year	0.978	0.930-1.029	0.392
Weight per 1 kg increment	0.989	0.949-1.031	0.598
Body mass index per 1 kg/m^2 increment	0.958	0.826-1.112	0.577
CD4 count per 1 /μl increment	1.001	0.997-1.004	0.628
Baseline eGFR per 1 ml/min/1.73 m^2 decrement	1.003	0.977-1.030	0.803
HIV-1 viral load per 1 log$_{10}$/ml increment	0.912	0.621-1.340	0.640
Hepatitis C infection	8.846	0.980-79.86	0.052
Hepatitis B infection	1.533	0.092-25.46	0.766
Treatment naïve	0.649	0.253-1.663	0.368
History of nephrolithiasis	3.333	0.572-19.44	0.181
Uric acid per 1 mg/dl increment	1.204	0.929-1.560	0.160
Co-administration of tenofovir	0.722	0.272-1.919	0.514
History of indinavir use	1.571	0.296-8.339	0.596
ABCB1			
193 A/A vs A/G or G/G	0.790	0.318-1.966	0.612
365 T/T vs T/C or C/C	2.523	0.634-10.03	0.189
1236 C/C vs C/T or T/T	0.712	0.218-2.327	0.573
2677 T/T vs T/A or G/G or G/T or G/A or A/A	1.615	0.426-6.124	0.481
3435 T/T vs T/C or C/C	2.067	0.509-8.401	0.310
NR1I2			
131 A/A vs A/C or C/C	1.018	0.401-2.584	0.971
370 G/G vs G/A or A/A	0.674	0.247-1.842	0.442
522 C/C vs C/T or T/T	0.742	0.270-2.041	0.563
1195 C/C vs C/A or A/A	0.702	0.296-2.271	0.702
1232 C/C vs C/T or T/T	0.674	0.247-1.842	0.442
44477 C/C vs C/T or T/T	1.064	0.424-2.673	0.894
63396 C/C vs C/T or T/T	2.173	0.451-10.46	0.333
UGT1A1			
211 G/G vs G/A or A/A	0.897	0.352-2.288	0.820
c.211 T/C vs C/C	**3.758**	**1.215-11.63**	**0.022**
339 G/C vs C/C	**5.912**	**1.675-20.87**	**0.006**
440 G/G or G/C vs C/C	**5.912**	**1.675-20.87**	**0.006**
SLCO1B1			
388 G/G vs G/A or A/A	1.571	0.296-8.339	0.596
521 T/T vs T/C or C/C	0.938	0.362-2.430	0.896
CYP3A5			
14 T/T vs T/C or C/C	0.939	0.379-2.327	0.891

eGFR: estimated glomerular filtration rate.

Table 4. Multivariate analysis to estimate the risk of SNP of UGT1A-3' UTR for ATV-induced nephrolithiasis.

SNP of UGT1A-3' UTR	Adjusted OR	95% CI	P value
Genotype T/C vs. C/C at c.211	**3.672**	**1.131-11.92**	**0.030**
Genotype G/C vs. C/C at 339	**5.763**	**1.557-21.33**	**0.009**
Genotype G/G or G/C vs. C/C at 440	**5.763**	**1.557-21.33**	**0.009**

Each variable was adjusted for sex, age, and hepatitis C infection.
UTR: untranslated region

Summary of the results

- ✓ 31 case patients with ATV-induced nephrolithiasis and 47 consecutive control patients who continued ATV/r for >2 years were enrolled.
- ✓ Table 1 listed the baseline characteristics. Sex, age, and established risk factors for nephrolithiasis (weight, BMI, serum uric acid, history of nephrolithiasis, and history of indinavir use) were not different between the two groups, except for hepatitis C infection, which was more common among case patients (p=0.034). Serum total bilirubin was higher in case patients than the controls (p<0.001).
- ✓ Table 2 summarizes the distribution of genotypes at the *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1*, and *CYP3A5* genes in the two groups. All polymorphisms were in Hardy-Weinberg equilibrium with a cut-off *p* value of 0.001.
- ✓ A higher percentage of patients with nephrolithiasis had genotype T/C vs. C/C at position c.211 (p=0.025), genotype G/C vs. C/C at 339 (p=0.007), and genotype G/G or G/C vs. C/C at 440 (p=0.011) of UGT1A-3'UTR. These three SNPs are in linkage disequilibrium with each other (D' >0.9).
- ✓ There was no difference in the distribution of other 16 SNPs in *ABCB1*, *NR1I2*, *SLCO1B1*, and *CYP3A5* between cases and controls.
- ✓ Univariate analysis showed a significant association between ATV-induced nephrolithiasis and genotype T/C vs. C/C at c.211 (OR=3.758; 95%CI, 1.215-11.63; p=0.022), G/C vs. C/C at 339 (OR=5.912; 95%CI, 1.675-20.87; p=0.006), and G/G or G/C vs. C/C at 440 (OR=5.912; 95%CI, 1.675-20.87; p=0.006) of UGT1A-3'UTR (Table 3).
- ✓ Multivariate analysis adjusted for sex, age, and hepatitis C infection identified genotype T/C at c.211 (adjusted OR=3.672; 95%CI, 1.131-11.92; p=0.030), G/C at 339 (adjusted OR=5.763; 95%CI, 1.557-21.33; p=0.009), and G/G or G/C at 440 (adjusted OR=5.763; 95%CI, 1.557-21.33; p=0.009) of UGT1A-3'UTR as independent risk factors for nephrolithiasis (Table 4).

Conclusion

✓To our knowledge, this is the first study that elucidated the association between genetic polymorphisms in the genes encoding proteins that affect ATV exposure and ATV-induced nephrolithiasis.
✓Japanese HIV-1-infected patients with variants in UGT1A-3'UTR were approximately 5 times more likely to develop ATV-induced nephrolithiasis, compared to those with wild types, who were well-matched for other traditional risk factors for nephrolithiasis.
✓Further studies are warranted to elucidate how these SNPs influence metabolism and excretion of ATV and formation of nephrolithiasis.
✓Larger studies are also needed to determine whether it is preferable to avoid or discontinue ATV in patients with these variants.

Reference:
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