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The pharmacokinetics of codeine and its metabolites in Blacks with sickle cell disease

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Abstract

Purpose—We conducted a prospective, open-label study in 54 adult subjects with sickle cell disease to determine the relationship between morphine concentrations, cytochrome P450 (*CYP*) 2D6 genotype, and clinical outcomes.

Methods—A blood sample was obtained for genotyping and serial blood samples were drawn to measure codeine and its metabolites in the plasma before and after oral codeine sulfate 30mg. Codeine and its metabolites were measured by liquid chromatography tandem mass spectrometry (LC-MS). *CYP2D6* genetic testing included four single nucleotide polymorphisms (SNP) indicative of three variant alleles: *17 (1023T); *29 (1659A, 3183A); and *41 (2988A) alleles.

Results—Thirty subjects (group I) had a mean (standard deviation) maximal morphine concentration of 2.0 (1.0) ng/ml. Morphine was not measurable in the remaining 24 subjects (group II). Nine (30%) subjects in group I and 11 (46%) subjects in group II carried a variant *17, *29, or *41 allele ($p=0.23$); one (3%) subject in group I and 5 (21%) subjects in group II were homozygous for *17 or *29 allele ($p=0.07$). Emergency room visits (group I 1.5 ± 1.8 vs. group II 2.1 ± 4.3 , $p=NS$) did not differ based on metabolic status, but more hospital admissions (0.9 ± 1.4 vs. 2.2 ± 4.1 , $p=0.05$) were documented in patients with no measurable morphine concentrations.

Conclusions—We conclude that Blacks with sickle cell disease without measurable plasma morphine levels after a single dose of codeine were not more likely to be a carrier of a single variant allele commonly associated with reduced *CYP2D6* metabolic capacity; however, homozygosity for a variant *CYP2D6* allele may result in reduced metabolic capacity. Furthermore, it appears that subjects without measurable morphine concentrations were more likely to be admitted to the hospital for an acute pain crisis.

Keywords

Sickle cell disease; Codeine; Morphine; Cytochrome P450 enzymes; Drug metabolism; Genotype

Introduction

Sickle cell disease is a group of inherited hemoglobin disorders commonly associated with frequent episodes of severe, debilitating pain. Codeine containing medications (CCM) are frequently prescribed to manage pain crises. However, some patients experience minimal relief with these drugs and require other opioids to manage their pain. Interpatient variability in responses to CCM is not well-understood.

Codeine undergoes *O*-demethylation by the polymorphic enzyme cytochrome P450 (*CYP*) 2D6 to the active metabolite morphine (5% to 10% of parent drug) [36]. (Fig. 1) Morphine strongly contributes to the analgesic, antitussive, and antidiarrheal effects commonly associated with CCM [8, 23, 32]. Morphine undergoes further metabolism to a 3- and 6-glucuronide; the 6-glucuronide demonstrates substantially greater analgesic effects compared to morphine. The 3-glucuronide contributes to the neuroexcitatory effects associated with morphine [8, 18, 23, 25, 26, 28–30, 32].

About 70 *CYP2D6* variant alleles along with additional subvariants are identified to date. The most common variant alleles associated with reduced activity in Blacks (Americans and Africans) following standard probe drugs are *CYP2D6**17 and *29, with reported frequencies ranging from 0.14 to 0.26 for *CYP2D6**17 and 0.08 for *CYP2D6**29 [9, 11, 16, 33, 34]. Two other reduced function alleles, *CYP2D6**10 and *CYP2D6**41 can be found in Blacks [4, 10, 11]. Their frequencies are approximately 0.03 and 0.02, respectively.

We propose that Blacks with sickle cell disease carrying *CYP2D6**17 or *29 alleles will report minimal or no analgesia with CCM and that these individuals may be more likely to seek medical care with the need for other opioids for acute pain management. Therefore, we conducted a prospective, open-label study to examine the relationship between morphine concentrations, *CYP2D6* genotype, and clinical outcomes in sickle cell disease. Positive associations between morphine concentrations, outcomes and genotype could lead to the

ability to predict response to CCMs based on genotype and individualize analgesic therapy for an acute pain crisis in sickle cell disease.

Patients and methods

Study design and population

This was a prospective, open-label study to examine the relationship between morphine concentrations, *CYP2D6* genotype, and clinical outcomes. The study population included people with sickle cell disease with the hemoglobin genotype SS who were prescribed CCM and received care at the Sickle Cell Center at the University of Illinois Medical Center (UIMC). Advertisements were posted within the medical center to identify interested individuals. The medical records of potential participants were reviewed to establish eligibility. The exclusion criteria were: contraindications to codeine; serum creatinine > 2.0 mg/dl; serum aspartate or alanine transaminases or direct bilirubin greater than three times the upper limit of normal; pregnancy, and a history of an acute pain crisis within two weeks of the study. Smoking, alcohol consumption, opioid analgesics, or medications known to inhibit *CYP2D6* were prohibited for at least 48 hours before the study visit. Study visits were rescheduled for all subjects with an acute pain crisis or the ingestion of CCM within 2 weeks or 48 hours, respectively, of the visit. The study was performed in the Sickle Cell Center (subjects 1 to 34) or the Clinical Research Center (CRC, subjects 35 to 58) at the UIMC, and was approved by the Scientific Advisory Committee for the CRC and the Institutional Review Board for the University of Illinois at Chicago.

Study procedures

Subjects who provided written informed consent were admitted to the Sickle Cell Center or the CRC for an 8-hour outpatient visit. No direct analgesic response was assessed. A venous blood sample was collected to determine *CYP2D6* genotype (EDTA-containing collection tube). Codeine sulfate 30 mg was administered with 200 ml of water after at least an 8 hour fast. Serial blood samples were collected 30 min before and 1, 2, 3, 4, and 6 hours after oral codeine intake (heparin containing collection tube). Blood for genotyping was stored at -80°C until analysis. Plasma for pharmacokinetic analysis was separated by centrifugation at 3,000 g for 15 min at 4°C , and samples were stored at -20°C . The number of emergency room (ER) visits and hospital admissions in the 12 months preceding the outpatient visit was determined by interviewing the subject and reviewing the medical record.

Measuring codeine, morphine, morphine-3-glucuronide and morphine-6-glucuronide

Codeine, morphine, M3G and M6G in plasma were measured by modifying a previously published liquid chromatography-tandem mass spectrometry method [14]. Plasma (200 μl) samples were spiked with internal standard (50 μl ; 200ng/ml of morphine- d_3 , 50ng/ml of M3G- d_3 , 50ng/ml codeine- d_3 in water) and prepared using a solid phase extraction method with Oasis HLB cartridges (Waters, Milford, MA). The analytes were eluted with 1 ml of methanol, evaporated to dryness by nitrogen at 40°C , and then reconstituted with 200 μl of 95% water and 5% methanol with 0.1% formic acid. Ten μl of the reconstituted material was injected onto a 5 μm Zorbax Eclipse XDB-C₁₈ 150 $\times$ 4.6 mm column (Agilent Technologies, Santa Clara, CA). The gradient was started with 95% mobile phase A (water with 0.1% formic acid) and 5% mobile phase B (methanol with 0.1% formic acid) pumped at a flow rate of 0.6 ml/min. From 0.5 to 5 min, a linear gradient was initiated to decrease mobile phase A from 95% to 10%, with 10% mobile phase A maintained for one min. From 6 to 6.1 min, a linear gradient was initiated to increase mobile phase A from 10% to 95%, with 95% mobile phase A maintained until the end of the run (10 min). Retention times for codeine, morphine, M3G and M6G, were 6.3, 5.7, 5.3 and 5.7 min, respectively.

MS analyses were performed on a 3200 Q TRAP triple quadrupole MS with a TurboIonSpray® source (Applied Biosystems, Foster City, CA). The positive ion mode was chosen and quantitation was performed in the multiple reaction mode (MRM) using nitrogen as the collision gas. Precursor-to-product ion transitions of m/z 286152 for morphine, m/z 462286 for M3G and M6G, and m/z 300165 for codeine were used for the MRM with dwell times of 120ms for analytes and 80ms for internal standards.

The analytes were quantified using a calibration curve ($r^2 > 0.99$) of peak area ratios of the opioid to the internal standard versus the analyte concentration. The lower limit of quantitation was 1 ng/ml for codeine and its metabolites. Samples with concentrations of opioids above the calibration curves were sufficiently diluted and reassayed. Interassay coefficients of variation were below 10% for all quality controls. The accuracy was between 96% and 107% for all opioids. Codeine, morphine and morphine- d_3 were purchased from Alltech (State College, PA) and codeine- d_3 , M3G, M6G, and M3G- d_3 were purchased from Cerilliant (Round Rock, TX).

Determining CYP2D6*17, CYP2D6*29 and CYP2D6*41 genotype

The DNA was extracted from whole blood using a Puregene® kit (Gentra Systems, Inc., Minneapolis, MS) and stored at -20°C . The *CYP2D6**17, *29 and *41 alleles were identified using polymerase chain reactions (PCR) followed by RFLP, bi-allelic discrimination or capillary sequencing. If none of these polymorphisms were observed, the allele assignment was defaulted to 'other'.

To detect the *CYP2D6**17 allele (1023C>T), a 199bp fragment of the *CYP2D6* gene spanning the polymorphic site of interest was amplified by PCR using the forward primer 5'-CTTCGGGGACGTGTTTCAG-3' and reverse primer 5'-CCCACGGAAATCTGTCTC-3'. The 25µl reaction mixture contained HotStarTaq™ Master Mix (Qiagen, Valencia, CA), 10µM of each forward and reverse primer, 20 to 100 ng/ml of genomic DNA and RNase free water. Thermocycling consisted of an initial denaturation for 15 min at 95°C , followed by 35 cycles (94°C for 30s, 60°C for 30s and 72°C for 1 min) and a final extension for 10 min at 72°C . These products were digested overnight with *HphI* restriction enzyme (New England Biolabs, Beverly, MA), and the resulting fragments were separated on an 8% polyacrylamide gel. Fragment sizes of 104 and 94 bp indicated the T allele and fragment sizes of 94 and 80 bp indicated the C allele. To verify assay specificity, samples from 6 subjects (2 non-carriers, 2 heterozygous carriers, and 2 homozygous carriers) were analyzed by capillary sequencing using the ABI PRISM 3100 Genetic Analyzer and BigDye Terminator™ technology (v 3.1, Applied Biosystems).

To detect the *CYP2D6**29 allele (1659G>A and 3183G>A), long range (XL) PCR was performed as previously described [9]. Subsequently, the 3183G>A SNP was detected by allele-specific PCR using the forward primer 5'-ATTGTGGGGACGCATGTCT-3' and reverse primer 5'-GGGACGATGTCCCCAAAG-3'. The PCR product was purified using a Qiaquick PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA) and 3183G>A was determined by capillary sequencing. All subjects positive for 3183A (i.e. the *CYP2D6**29 allele) were confirmed by testing for 1659G>A also by sequence analysis of purified PCR product.

To detect the *CYP2D6**41 allele (2988G>A), bi-allelic discrimination was completed using an inventoried Taqman SNP Genotyping Assays (Applied Biosystems). Reactions were carried out in a final volume of 5µl following the protocol provided. The assay was performed in duplicate for each sample and non-template control used for each assay. Endpoint bi-allelic discrimination was performed using the ABI Prism 7900HT SDS.

Pharmacokinetic analysis

Non-compartmental analysis was used to estimate the pharmacokinetic parameters for codeine, morphine, M3G and M6G using the measured concentrations and actual times with the aid of the program WinNonlin® Professional (v. 4.0, Pharsight Corp., Mountain View, CA). Log-linear regression of the terminal slope was used to estimate the elimination rate constant (k) and the elimination half-life ($t_{1/2}$) was calculated by the equation $t_{1/2} = 0.693/k$. The apparent peak plasma concentration ($C_{\max}$) for codeine and its metabolites was visually identified from the plasma concentration time curve. The area under the concentration-time curve from 0 to $t=6$ h (AUC_{0-6}) was calculated using the log-linear trapezoidal rule. The $AUC_{0-\infty}$ was calculated by adding the AUC_{0-6} to the terminal area determined by dividing the last measurable plasma concentration by k . The apparent oral clearance (Cl_o) was calculated from the equation $Cl_o = \text{dose}/AUC_0$.

Statistical analysis

The primary outcome measure was *CYP2D6* genotype and the secondary outcome measures were ER visits and hospital admissions and the concentrations of the glucuronide metabolites. The mean and standard deviation is reported for all continuous data and the number and percentage is reported for all categorical data. The outcome measures were compared between metabolic status by two sample Wilcoxon test and Fisher Exact test. P-value < 0.05 was considered statistical significant. All of the data were analyzed with the aid of the statistical program SAS (version 9.1.3, SAS Institute, Inc., Cary, NC).

Results

Fifty-four subjects completed the study (Table 1). Morphine was not measurable in the plasma of 24 subjects (group II), whereas morphine was measurable in the remaining 30 subjects (group I). The maximal codeine, morphine, M3G, and M6G concentrations are provided in Table 2. The time to the maximal concentration was delayed, and the maximal codeine concentrations ($C_{\max}$) were lower in subjects in group II ($P < 0.05$) (Fig. 2). Not surprisingly, the concentrations of M3G and M6G were lower in subjects in group II based on the absence of morphine concentrations ($P < 0.001$). The time to the maximal concentrations of either glucuronide was similar between the groups.

The number of carriers of a variant *CYP2D6* allele did not significantly differ between group I and group II subjects. (Table 3). Nine carriers of a variant allele were identified in group I and 11 carriers of a variant allele were identified in group II ($p = 0.23$). More *CYP2D6* variant allele homozygotes were identified in group II compared to group I (group I 1 vs. group II 5, $p = 0.07$).

The mean pharmacokinetic parameters estimated for codeine and its metabolites were compared between the 12 carriers of the *17 allele and the 34 non-carriers of a *17 allele, with non-carriers designated as *other/*other. The mean $C_{\max}$ and AUC_{0-6} estimated for morphine was 40% and 30% lower among variant allele carriers, respectively, but the differences did not reach statistical significance. However, we observed significantly lower mean $C_{\max}$ and AUC_{0-6} for M3G and M6G in *17 carriers versus noncarriers ($p < 0.05$) (Fig. 3). Similarly, the $C_{\max}$ estimated for codeine and its metabolites tended to be lower in *29 allele carriers ($n = 7$) compared to non-carriers ($p = 0.07$).

The mean number of ER visits and hospital admissions in the 12 months preceding the study visit was higher for subjects in group II compared to group I (Table 1). Emergency room visits (group I 1.5 ± 1.8 vs. group II 2.1 ± 4.3 , $p = \text{NS}$) did not differ based on metabolic status, but more hospital admissions (0.9 ± 1.4 vs. 2.2 ± 4.1 , $p = 0.05$) were documented in patients with no measurable morphine concentrations.

Discussion

We examined the relationship between morphine concentrations, *CYP2D6* genotype, and clinical outcomes in 54 Blacks with sickle cell disease. We found that the variant *CYP2D6* allele frequency was significantly higher in patients without measurable morphine concentrations following a dose of codeine. In addition, carriers of two variant *CYP2D6* alleles tended to be overrepresented in the group without measurable morphine. Furthermore, the concentrations of morphine or its metabolites tended to be lower in carriers of a variant *17 or *29 allele. These differences did not extend to significant differences in ER visits, but more hospital admissions were documented in subjects without measurable morphine concentrations.

We opted not to label our subjects as poor metabolizers (PM) or extensive metabolizers (EM), since codeine is not a standard probe drug in which phenotype has been predicted based on a well-defined plasma or urinary metabolic ratio. Early systems used to predict phenotype distinguished between PM and EM using a urinary metabolic ratio of a standard probe drug, such as dextromethorphan or debrisoquine [13]. Two studies indicate that less morphine, M3G, and M6G are measurable in Whites identified as PM based on genotype or phenotype [5, 35]. However, these data should be extrapolated to Black populations with caution. Black population samples demonstrate slower *CYP2D6* activity compared to Whites that is evidenced as a 'right-shift' in the activity distribution and higher mean metabolic ratios. More over, substantial differences have also been observed among Black American and African populations [2, 9, 17, 20, 21].

We examined the *17 and *29 alleles, because these are the most common variant *CYP2D6* alleles reported in Blacks, and these alleles are associated with a reduced metabolic capacity using standard probe drugs [3, 11, 27, 31, 34]. Furthermore, two reports indicate that *17 allele is associated with reduced metabolic capacity for codeine [27, 31]. Oscarson et al. [27] reported that the *17 allele is associated with 5- to 10-lower affinity for codeine compared to the wild-type allele and Shen et al. [31] reported that the intrinsic clearance of codeine was reduced by ~20%. Our data also suggests that *17 allele is associated with reduced metabolic capacity, since the C_{max} and AUC_{0-6} of morphine, M3G and M6G were substantially lower for *17 carriers. However, this SNP is also part of different haplotypes which have been found in a number of other allelic variants including *CYP2D6**40. This *40 allele is associated with a loss of function and occurs at a frequency 0.006 in Blacks [9]. Therefore, it is conceivable that a fraction of our twelve *CYP2D6**17 alleles have been misclassified. Although the metabolic capacity of the *17 and *40 alleles is substantially different, a misclassification is not likely to affect the outcomes of our study with likelihood only one subject in our study may be a carrier of the *40 allele. The affinity and intrinsic clearance for codeine has not been described for *29 allele, but our data appears to indicate that codeine *O*-demethylation is reduced in *29 carriers.

Of note, the sensitivity of our assay is in agreement with other LC-MS assays using similar equipment [19, 24]. A more sensitive assay would likely lead to more subjects in our study with measurable morphine concentrations and reclassified into group I; the number of subjects that could be reclassified cannot be predicted. However, our mean serum molar ratio of M3G to morphine, M6G to morphine and M3G to M6G for subjects in group I and the M3G to M6G in subjects in group II agrees with previous published reports in which subjects received oral morphine [1, 12, 15].

Interestingly, the mean C_{max} and AUC for M3G and M6G were substantially lower for *17 carriers. *CYP2D6* catalyzes the *O*-demethylation of codeine to morphine, and the polymorphic enzyme uridine diphosphate-glucuronosyltransferase (UGT) 2B7 catalyzes the

metabolism of morphine to M6G and possibly to M3G [6, 7]. Therefore, it can be argued that the rate-limiting step in the metabolism of codeine to morphine to its glucuronides is the metabolism of codeine to morphine; this would support the relationship between *CYP2D6* genotype and the concentrations of the glucuronide metabolites. We also noted that mean maximal codeine concentrations were lower in subjects in group II compared to subjects in group I which is contrary to our expectations. It is not apparent why the mean maximal codeine concentrations were lower in this group; we would anticipate that maximal codeine concentrations would be higher in the subjects in group II secondary to reduced metabolism to morphine. However, only about 10% of codeine undergoes *O*-demethylation by the polymorphic enzyme *CYP2D6*. Codeine predominantly undergoes metabolism by *CYP3A4* to norcodeine. Both norcodeine and the remaining metabolites undergo further metabolism by the phase II enzyme *UGT*. It is possible inhibition or reduced function alleles of *CYP3A4* or *UGT* may be more common in the subjects in group I compared to group II and led to a statistically significant increase in the mean maximal codeine concentration in the subjects assigned to group I. Alternatively, it appears that the orocecal transit time following codeine is prolonged in extensive metabolizers of *CYP2D6* [22]. The prolonged transit time would allow for increased absorption and could account for the higher mean maximal plasma concentration of the parent drug codeine in our subjects assigned to group I.

In this study population, we identified a relationship between metabolic status and hospital admissions, but not ER visits. ER visits and hospital admissions for an acute pain crisis were quantified as a clinical surrogate marker of response to codeine. A more direct measure of response, such as heat intolerance, cold intolerance and pinprick response, was not used in this study, because these techniques could elicit a pain crisis. An indirect measure of a response to codeine was therefore the best estimation of treatment response and provided a non-invasive opportunity to explore the relationship between genotype, phenotype and outcomes. Our failure to demonstrate a relationship between the ER visits and phenotype may be due to the use of a poor surrogate marker for clinical response to codeine; this marker is likely influenced by a large number of factors that could wash out any possible influence of phenotype.

In summary, the data suggest that subjects with sickle cell disease with no measurable plasma morphine levels after a single dose of codeine were not more likely to possess a variant allele commonly associated with reduced *CYP2D6* metabolic capacity in Blacks. However, we did observe differences in the frequency of heterozygotes and homozygotes for some variants between subjects with and without measurable morphine concentrations and carriers of these alleles demonstrated reduced concentrations of morphine or its metabolites. It is possible the observed relationship may be clinically relevant, although we did not identify a statistically significant relationship with the both surrogate markers. Additional prospective studies are warranted to determine if clinical response to codeine or hydrocodone may be predicted based on phenotype.

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Fig. 1.

The metabolism of codeine. Each of the opioids in the pathway may undergo further metabolism to form one or more glucuroindes. The structures are courtesy of ChemSpider: a database of chemical structures and property predictions (www.chemspider.com)

Fig. 2.

The mean (and standard deviation) plasma concentration time profile for the 30 subjects in group I (●) and the 24 subjects in group II (○) are illustrated. The whiskers represent the standard deviation

Fig. 3.

The mean maximal plasma concentrations for codeine, morphine, morphine-3-glucuronide and morphine-6-glucuronide are illustrated in non-carriers and carriers of the three variant alleles identified in this study population. The whiskers represent the standard deviation

Table 1

Study population

Characteristic	All (n=54)	Group I (n=30)	Group II (n=24)
Age, years (mean (stdev))	32 (12)	32 (12)	31 (14)
Gender (n (%))			
Men	18 (33)	9 (30)	9 (38)
Women	36 (67)	21 (70)	15 (62)
Medications (n (%))			
Folate	44 (81)	26 (87)	18 (75)
Hydroxyurea	32 (60)	15 (50)	17 (70)
Co-existing illness (n (%))			
Acute chest syndrome	7 (13)	3 (10)	4 (17)
Avascular necrosis	6 (11)	2 (7)	4 (17)
Cerebrovascular accident	8 (15)	4 (13)	4 (17)
Cholecystectomy	15 (28)	9 (30)	6 (25)
Chronic renal insufficiency	4 (7)	2 (7)	2 (8)
Priapism (men only)	3 (6)	2 (22)	1 (14)
Pulmonary hypertension	4 (7)	3 (10)	1 (4)
Skin ulcers	6 (11)	5 (17)	1 (4)
Visits or admissions documented (mean (stdev))			
Emergency room	1.7 (3.1)	1.5 (1.8)	2.1 (4.3)
Hospital admission ^a	1.5 (2.9)	0.9 (1.4)	2.2 (4.1)

^aP<0.05

Table 2

Mean (stdev) pharmacokinetic parameters for codeine and its metabolites based on metabolic status

	Group I (n=30)	Group II (n=24)
Codeine		
$t_{1/2}$ (min)	125 (48)	120 (27)
T_{max} (min) ^a	54 (27)	77 (36)
C_{max} (ng/ml) ^a	81 (32)	62 (24)
$AUC_{(0-\infty)}$ ($\mu\text{g}\cdot\text{min}/\text{ml}$)	22 (23)	14 (6.4)
Morphine		
T_{max} (min)	46 (25)	–
C_{max} (ng/ml)	2.0 (1.0)	0.0
$AUC_{(0-\infty)}$ ($\text{ng}\cdot\text{min}/\text{ml}$)	222 (246)	0.0
Morphine-3-glucuronide		
T_{max} (min)	104 (69)	110 (52)
C_{max} (ng/ml) ^a	88 (38)	38 (21)
$AUC_{(0-6)}$ ($\mu\text{g}\cdot\text{min}/\text{ml}$) ^a	22 (10)	9.2 (5.3)
Morphine-6-glucuronide		
T_{max} (min)	122 (80)	126 (40)
C_{max} (ng/ml) ^a	9.5 (3.8)	8.3 (4.5)
$AUC_{(0-6)}$ ($\text{ng}\cdot\text{min}/\text{ml}$) ^a	2185 (911)	1017 (504)

^aP<0.05

Table 3

Allele and genotype frequencies in Blacks with sickle cell disease

	Frequencies All	Group I	Group II
Allele	n=108	n=60	n=48
*17	17 (0.16)	7 (0.12)	10 (0.21)
*29	8 (0.07)	2 (0.03)	6 (0.12)
*41	1 (0.01)	1 (0.02)	0 (0.0)
*other	82 (0.76)	50 (0.83)	32 (0.67)
Genotype	n=54	n=30	n=24
*other/*other	34 (0.63)	21 (0.70)	13 (0.54)
*other/*17	7 (0.13)	5 (0.17)	2 (0.08)
*other/*29	6 (0.11)	2 (0.07)	4 (0.17)
*other/*41	1 (0.02)	1 (0.03)	0 (0.0)
*17/*17	5 (0.09)	1 (0.03)	4 (0.17)
*29/*29	1 (0.02)	0 (0.0)	1 (0.04)

Values are numbers with proportion in parenthesis