Association of the Multidrug Resistance-1 Gene Single-Nucleotide Polymorphisms with the Tacrolimus Dose Requirements in Renal Transplant Recipients

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Abstract. The immunosuppressive drug tacrolimus, whose pharmacokinetic characteristics display large interindividual variations, is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance-1 (MDR1) gene. Some of the single nucleotide polymorphisms (SNP) of MDR1 reported correlated with the in vivo activity of P-gp. Because P-gp is known to control tacrolimus intestinal absorption, it was postulated that these polymorphisms are associated with tacrolimus pharmacokinetic variations in renal transplant recipients. The objective of this study was to evaluate in a retrospective study of 81 renal transplant recipients the effect on tacrolimus dosages and concentration/dose ratio of four frequent MDR1 SNP possibly associated with P-gp function (T-129C in exon 1b, 1236C>T in exon 12, 2677G>T in exon 21, and 3435C>T in exon 26). As in the general population, the SNP in exons 12, 21, and 26 were frequent (16, 17.3, and 22.2% for the variant homozygous genotype, respectively) and exhibited incomplete linkage disequilibrium. One month after tacrolimus introduction, exon 21 SNP correlated significantly with the daily tacrolimus dose (P ≤ 0.05) and the concentration/dose ratio (P ≤ 0.02). Tacrolimus dose requirements were 40% higher in homozygous than wild-type patients for this SNP. The concentration/dose ratio was 36% lower in the wild-type patients, suggesting that, for a given dose, their tacrolimus blood concentration is lower. Haplotype analysis substantiated these results and suggested that exons 26 and 21 SNP may be associated with tacrolimus dose requirements. Genotype monitoring of the MDR1 gene reliably predicts the optimal dose of tacrolimus in renal transplant recipients and may predict the initial daily dose needed by individual patients to obtain adequate immunosuppression.

Tacrolimus, like cyclosporine, is a member of the calcineurin inhibitor family. It is used as an alternative to cyclosporine to prevent allograft rejection in solid organ transplantation and therefore is a basic component of immunosuppressive regimens for renal transplant recipients. Tacrolimus is a critical-dose drug with a narrow therapeutic index. Moreover, its pharmacokinetic characteristics may vary greatly among individuals, and daily doses must be adjusted according to the whole-blood trough tacrolimus concentrations measured 12 h post-dose, just before the next dose. Achieving therapeutic trough levels is of critical importance during the initial period after transplantation, when the risk of rejection is greatest. Identification of the parameters that are predictive of the optimal tacrolimus dosage might be clinically important for rapid determination of the adequate therapeutic concentration.

Tacrolimus is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance (MDR1) gene (1). P-gp acts as a transmembrane efflux pump involved in energy-dependent export of xenobiotics from inside the cells. It is present in intestinal epithelial cells, in biliary canicular cells, in the blood-brain barrier, in lymphocytes, and on the luminal surface of proximal tubule kidney cells (2–4). P-gp affects the absorption of drugs from the gut and their distribution among the body’s compartments and also their metabolism and excretion (5). In the gut, strong expression and/or function of P-gp lowers the substrate’s absorption. Conversely, alteration in expression and/or function raises this drug absorption.

Previous data for liver transplant recipients who were treated with tacrolimus have demonstrated that intestinal mRNA expression of the MDR1 gene is inversely correlated to the concentration/dose ratio, which expresses the tacrolimus dose needed to obtain a given blood concentration (6). In this study, patients with strongly expressed MDR1 required higher tacrolimus doses to achieve trough concentrations similar to those of patients with weak expression. These results suggested that...
P-gp expression plays a critical role in the ability of the gut to absorb tacrolimus.

Significant interindividual variations in the expression and function of P-gp may be due to genetic factors. Various single nucleotide polymorphisms (SNP) have been identified within the MDR1 gene in the past 3 yr (7–17). In particular, one SNP, located in exon 26 (exon 26 3435C>T), has been studied extensively. When first described, this SNP was associated with variations in intestinal expression and function of P-gp (7). This expression was stronger for the 3435C/C allele than for the 3435T/T allele, and the area under the plasma concentration curve for orally administered digoxin was larger for the 3435T/T allele than for the 3435C/C allele (7). Several groups have reported that this SNP is associated with decreased MDR1 expression in CD56+ natural-killer cells (15) and placental tissues (12). However, other reports have led to contradictory results regarding the effect on this SNP in phenotypic consequences or P-gp protein expression. Von Ahsen et al. (16) found no association between this SNP and the dose and efficacy of cyclosporin A, another P-gp substrate, and Kim et al. (17) reported a decrease in fexofenadine with the T allele of the synonymous exon 12 SNP C1236T (exon 12 T). A noncoding SNP located in the promoter of the SNP. Because SNP C3435T is a silent polymorphism that does not result in any amino acid changes, the associations and discrepancies described suggest that it may be in linkage disequilibrium with other functional polymorphisms within the MDR1 gene. In white individuals, Kim et al. (17) indeed reported the co-segregation of exon 26 3435T with the T allele of the nonsynonymous exon 21 G2677T SNP (exon 21 2677G>T), resulting in an A893S amino acid change, and with the T allele of the synonymous exon 12 SNP C1236T (exon 12 1236C>T). A noncoding SNP located in the promoter of MDR1 (T-129C) has also been associated with weaker expression of P-gp in human placenta (12). Taken together, the association of several SNP led to haplotype analysis of the MDR1 gene to identify the links between the genomic variations, represented by each haplotype on the one hand and by altered MDR1 function on the other (11,13,14).

Because these polymorphisms may partly explain the large interindividual variations in the pharmacokinetic characteristics of tacrolimus and may control the extent of its uptake after ingestion, we investigated the effect of the four MDR1 SNP (exon 1b T-129C, exon 12 1236C>T, exon 21 2677G>T,A, and exon 26 3435C>T) most frequently suggested to be associated with P-gp function on tacrolimus doses and trough levels in renal transplant recipients (Figure 1).
CYP3A and/or P-gp interaction. The daily dose of tacrolimus (mg) was recorded, and the weight-adjusted tacrolimus dosage (mg/kg per d) was calculated. Laboratory evaluations included the tacrolimus blood trough concentrations (ng/ml). Blood tacrolimus levels were assayed 12 h after the previous dose, using the semiautomated microplate enzyme immunoassay (Abbott, Rungis, France). For reducing intraindividual variability, the tacrolimus blood trough concentration was calculated as the mean of three consecutive measurements. The concentration measured was dose-normalized using the concentration/dose ratio, obtained by dividing the tacrolimus trough concentration by the corresponding 24-h dose, on an mg/kg basis. The information thus obtained was the tacrolimus dose needed to obtain a given trough level.

**Identification of Genotypes**

For genotype determination, genomic DNA was extracted from EDTA-treated blood using the Qiagen Kit (Courtabeuf, France). MDR1 exon 26 (C3435T) single nucleotide polymorphism was detected by 5′ nucleic acid discrimination assay (ABI PRISM 7700 Sequence Detection System; Applied Biosystems, Foster City, CA) using the forward 5′-ATGTATGTGCTCCCTTTGCT-3′ and reverse 5′-AACAGCGGTGTTGTCA-3′ primers for amplification. Specific probes for each allele (5′-CTTCACGATCTCT-3′ and 5′-CCTCACAATCTCTT-3′) were respectively labeled with the fluorescence reporter dyes FAM and VIC at their 5′ extremities.

MDRI exon 1b, 12, and 21 SNP were detected by direct sequencing. The following primers were used for PCR amplification: exon 1b forward, 5′-TGTGTTGCTGGCGAAGACACG-3′; exon 1b reverse, 5′-AACATTTAGTCTCTTCCAGGCT-3′; exon 12 forward, 5′-TGATTGGCTGGGAGGAGAACAG-3′; exon 12 reverse, 5′-AATCTTGGAAGAAGATACTCC-3′; exon 21 forward, 5′-TCTGATTGACCGAGTCTCTCGC-3′; exon 21 reverse, 5′-AGGGTTATACAGATCT-3′; and exon 21 reverse, 5′-TGAGGAGTGGTTAAACACAT-3′. PCR were performed in a total volume of 25 μl using 100 ng of genomic DNA with 0.2 μM of each primer (0.4 μM for exon 1b), 0.2 mM dNTP, 1× PCR buffer, 2.5 mM MgCl2 for exon 1b or 2 mM MgCl2 for exon 12 and 21, and 0.625 units of AmpliTaq Gold DNA-polymerase (Perkin-Elmer, France). PCR products were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) on an ABI PRISM 3100 sequencer (Applied Biosystems).

**Statistical Analyses**

For analysis of continuous pharmacologic variables, patient genotypes were used as categorical independent variables. For the analyses of daily tacrolimus doses, blood tacrolimus levels, and tacrolimus concentration/dose ratios, groups were compared using nonparametric tests. We used the Mann-Whitney U test for comparisons between two groups and the Kruskal-Wallis test for comparisons among several groups. Allele and genotype frequencies for the various SNP were assessed for deviation from Hardy-Weinberg equilibrium using Fisher exact test. P < 0.05 was considered significant.

The different genotypes for the G2677T,A SNP were classified as follows: wild type (G/G), heterozygous (G/T or G/A), and homozygous for the variant (T/T, T/A, or A/A). Haplotype frequencies were estimated on the basis of the Expectation-Maximization algorithm (18) using the population genetics data analysis program Arlequin (www.lgb.unige.ch/arlequin/).

**Results**

**Frequency of MDR1 Variants in Renal Transplant Patients**

Table 1 shows the frequency of the four MDR1 SNP for each allele in the 81 renal transplant recipients. The frequency for each genotype was evaluated on the basis of Hardy-Weinberg equilibrium proportions. None of the observed frequencies was significantly different from the expected frequencies. In our population, the presence of the homozygous variant in the promoter region (exon 1b) was not observed, but nine patients were heterozygous for the mutant allele. A high frequency was observed for variants leading to an amino acid modification in exon 21 G2677T,A (Ala893Ser or Ala893Thr). More than 50% of the patients exhibited a mutated nucleotide at position 2677, and 17.3% were homozygous for the mutation. The other exonic variants, C1236T in exon 12 and C3435T in exon 26, which do not affect the amino acid sequence, were also frequently observed. The mutations were present homozygously in 16 and 22.2% of patients for exons 12 and 26, respectively.

**Linkage Disequilibrium Study**

We investigated the four SNP for any linkage disequilibrium to determine whether they were randomly associated in the same patient. Pairwise linkages between SNP were not randomly distributed, and only a few major linkages were observed (defined as >10% occurrence; Figure 2). It is interest-

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Effect</th>
<th>Nucleotide Sequence</th>
<th>Genotype* (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild Type (wt)</td>
<td>Mutation (m)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wt/wt</td>
<td>wt/m</td>
</tr>
<tr>
<td>T-129C</td>
<td>Noncoding</td>
<td>cagagTaccgc</td>
<td>72 (88.9%)</td>
</tr>
<tr>
<td>C1236T</td>
<td>wobble</td>
<td>aggacTcctga</td>
<td>29 (35.8%)</td>
</tr>
<tr>
<td>G2677TA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2677T</td>
<td>Ala893Ser</td>
<td>aggtGcttgg</td>
<td>26 (32.1%)</td>
</tr>
<tr>
<td>G2677A</td>
<td>Ala893Thr</td>
<td>aggtGcttgg</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>C3435T</td>
<td>wobble</td>
<td>agatCtctga</td>
<td>29 (35.8%)</td>
</tr>
</tbody>
</table>

* The frequency expected for each genotype was evaluated on the basis of Hardy-Weinberg equilibrium proportions. None of the observed frequencies was significantly different from the expected frequencies.
ing that the association of exon 1-129C with exon 12 1236T was never observed. All nine patients who harbored the C allele in exon 1 SNP also had the C allele in exon 12 SNP. Significant linkage disequilibrium was also observed between the exon 12, 21, and 26 SNP. Most of the wild-type alleles at any one position were associated with the wild-type allele at the following position. Conversely, a mutant allele in the exon 12 SNP was usually associated with mutant alleles in the exon 21 and 26 SNP. Comparison of the linkages between the four SNP with the correlation coefficient \( r \) showed that the T-129C SNP was not significantly associated with the genotypes at any of the positions of the three other SNP. The C1236T SNP correlated with the G2677T,A and C3435T SNP (\( r = 0.79 \) and 0.66, respectively), and the G2677T,A SNP correlated with the C3435T SNP (\( r = 0.66 \)).

![Figure 2. Pairwise linkage profiles of the four SNP of the MDR1 gene. The frequency of the different pairwise linkages was calculated on the basis of Expectation-Maximization (24). Dotted lines represent weak linkages (<10%), and solid lines represent strong linkages (frequency >10%). The number beside each line indicates the percentage of the specific linkage.](image)

**Effects of MDR1 SNP on Tacrolimus Dose Requirement**

We examined the relationship between each SNP of the MDR1 gene and the tacrolimus daily dose and concentration/dose ratio (Table 2). One month after tacrolimus introduction, there was no difference between the blood trough levels of the different MDR1 genotypes, confirming that the daily dose was adjusted to reach target tacrolimus blood level. As shown in Figure 4, 1 mo after tacrolimus introduction, the exon 21 SNP of 2677G>T,A SNP was significantly associated with the tacrolimus daily dose (\( P \leq 0.05 \), Kruskal-Wallis test). The mean dose required to obtain the target trough concentration was significantly higher in patients with the wild-type genotype than in those with one or two mutant alleles (0.20 ± 0.07 versus 0.16 ± 0.07, and 0.14 ± 0.07 mg/kg per d; \( P = 0.05 \)). Tacrolimus dose requirements were not significantly different for exons 1b, 12, and 26.

The average concentration/dose ratio correlated with the exon 21 2677G>T,A SNP. The concentration/dose ratios were respectively 65, 94, and 101 (ng/ml)/(mg/kg) for the wild-type, heterozygous, and homozygous variants (\( P = 0.01 \)). The average concentration/dose ratio also correlated with the C1236T SNP (\( P \leq 0.05 \)). Note that a similar nonsignificant trend was observed for the C3435T SNP (Table 2). The mean daily dose and concentration/dose ratio were also related to the number of mutant copies for each SNP.

**Haplotype Analysis**

There is increasing evidence that gene-based haplotype approaches that take into account the combination of SNP present in an allele make it easier to predict changes in response to drugs than SNP-based approaches (15,16). The in vivo effects of isolated mutations are difficult to evaluate because of the high frequency of the SNP and the close linkage disequilibrium detected between the exon 12, 21, and 26 SNP. This prompted us to perform a multipoint haplotype determination of the MDR1 gene to identify the associations between the genomic

**Figure 3. Distribution histograms of tacrolimus daily doses required to obtain the target trough level 1 mo after tacrolimus initiation in 81 renal transplant patients.**

**Figure 4.**
Table 2. Relationship between the four SNP tested and tacrolimus daily doses and concentration/dose ratios after 1 mo of tacrolimus treatment in 81 renal transplant recipients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Genotype</th>
<th>n</th>
<th>Daily Dose (mg/kg/d)</th>
<th>Concentration/Dose Ratio (ng/ml)/(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-129C</td>
<td>T/T</td>
<td>72</td>
<td>0.16 ± 0.07</td>
<td>89 ± 45</td>
</tr>
<tr>
<td></td>
<td>T/C</td>
<td>9</td>
<td>0.19 ± 0.07</td>
<td>65 ± 33</td>
</tr>
<tr>
<td>C1236T</td>
<td>C/C</td>
<td>29</td>
<td>0.19 ± 0.08</td>
<td>71 ± 38*</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>39</td>
<td>0.16 ± 0.07</td>
<td>91 ± 46*</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>13</td>
<td>0.14 ± 0.07</td>
<td>103 ± 48*</td>
</tr>
<tr>
<td>G2677T, A</td>
<td>G/G</td>
<td>26</td>
<td>0.20 ± 0.07*</td>
<td>65 ± 30*</td>
</tr>
<tr>
<td></td>
<td>G/mutant</td>
<td>41</td>
<td>0.16 ± 0.07*</td>
<td>94 ± 47*</td>
</tr>
<tr>
<td></td>
<td>mutant/mutant</td>
<td>14</td>
<td>0.14 ± 0.07*</td>
<td>101 ± 50*</td>
</tr>
<tr>
<td>C3435T</td>
<td>C/C</td>
<td>29</td>
<td>0.19 ± 0.07</td>
<td>76 ± 46</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>34</td>
<td>0.16 ± 0.07</td>
<td>88 ± 41</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>18</td>
<td>0.15 ± 0.07</td>
<td>98 ± 48</td>
</tr>
</tbody>
</table>

*P ≤ 0.05; *P ≤ 0.02.

The three genotype groups were compared using the Kruskal-Wallis test.

Discussion

The pharmacogenetic evaluation of several genes implicated in drug pharmacokinetics should provide efficient tools for individualizing drug therapy by optimizing drug dosage. This would both improve drug efficacy and prevent adverse effects (19). Such evaluation may be particularly useful for drugs characterized by a narrow therapeutic index and/or significant toxicity, such as the calcineurin inhibitors tacrolimus and cyclosporine. In this study, we demonstrated that several polymorphisms of the MDR1 gene partly explain the variability of tacrolimus dose requirement in renal transplant recipients.

Abundantly expressed in the enterocytes, P-gp, the MDR1 gene product, may play an important role in the variability of tacrolimus absorption (7,20–23). Thus, interindividual variations in P-gp expression and/or function may explain the interindividual variability of tacrolimus bioavailability among individuals. Genetic polymorphisms related to the intestinal expression of P-gp therefore may affect the uptake of tacrolimus in transplant patients. The correlations between MDR1 SNP and tacrolimus requirements may be interpreted as an enhanced uptake by the intestine of tacrolimus in the mutant carriers. Patients with no mutation in the MDR1 gene are more likely to extrude tacrolimus from intestinal cells and therefore need a higher daily dose to achieve adequate blood tacrolimus levels. Conversely, low expression of intestinal P-gp may reduce the P-gp–mediated drug efflux that directs intestinal secretion of drug into the gut lumen, resulting in greater tacrolimus bioavailability. Although the gut is the likely source, we cannot exclude that the SNP action could be occurring elsewhere. Because P-gp is located mainly in the apical membrane of excretory cells in the liver, kidney, and intestine (2,3), its expression is important for the absorption, distribution, and elimination of xenobiotics. Functional polymor-
Phenotypes in MDRI gene may then alter these pharmacologic parameters. However, a recent study showing an association between haplotypes of the MDRI gene and the steady-state kinetics of the P-gp substrate digoxin showed that MDRI polymorphisms were significantly associated with the absorption but not with the elimination of the drug, suggesting that the SNP action occurred mainly in the gut (24).

Our patients exhibited genotype frequencies of the different SNP in accordance with those already described in white populations. For example, Cascorbi et al. (25) in a genetic analysis of 461 German volunteers reported frequencies for the exon 12 1236C/H11022T polymorphism of 34.4, 49.2, and 16.4 for the C/C, C/T, and T/T genotypes, respectively. For the exon 21 SNP, 30.9% of their subjects were homozygous for the wild-type allele, 51.2% were heterozygous, and 17.9% exhibited two copies of mutant alleles. For the exon 26 polymorphism, the reported frequencies reveal that almost half of the individuals were heterozygous and 25 to 30% were homozygous carriers for the variant allele. These three polymorphisms therefore are found frequently in the general population, and their putative role in drug disposition may have a relevant impact in clinical practice.

The linkage that we found between the SNP in exons 12, 21, and 26 has already been reported in other populations (12,17). In European Americans, Kim et al. (17) reported co-segregation of the exon 26 mutant allele with the T allele of the nonsynonymous exon 21 SNP G2677T and with the T allele of the synonymous exon 12 SNP C1236T. These three SNP in exon 12, 21, and 26 are closely linked at high frequency and occurred in 62% of European Americans and 13% of African Americans. This high level of incomplete disequilibrium is at the origin of the haplotype approach. Haplotypes generally contain more information than did individual SNP (26,27).

Our results confirmed the large interindividual variations in tacrolimus pharmacokinetics. After 1 mo of tacrolimus treatment, a 1- to 12-fold range of doses is needed to achieve therapeutic concentrations. Our results suggest that genetic polymorphisms may explain a significant part of this variation.
in tacrolimus bioavailability after oral administration. Exon 1-129T>G mutations have been associated with P-gp expression (12). However, in accordance with previous authors (14), we found no phenotypic correlation with tacrolimus pharmacokinetics for this mutation. However, we found that tacrolimus dose requirements were generally lower in patients with one or two mutant alleles in their exon 12, 21, and 26 SNP, who therefore exhibited a gene-dose effect. The most important relation was noted for the exon 21 2677G>T SNP, which included the exon 26 SNP, suggesting that these two exons 12 and 26 SNP are strongly associated with the exon 21 SNP: haplotype 1, C-G-C; haplotype 2, T-T, A-T.

The three genotype groups were compared using the Kruskal-Wallis test. A nonsignificant trend was also observed with SNP in exon 12 and 26, which did not reach statistical significance. However, the most important relation was noted for the exon 21 2677G>T, A-T SNP, as the tacrolimus dose requirement was 40% higher in homozygous than in wild-type carriers. Similarly, the concentration/dose ratio was 36% lower for this mutation in wild-type patients, suggesting that for a given dose, the tacrolimus blood concentration is lower in the wild-type patients. A similar nonsignificant trend was also observed with SNP in exon 12 and 26. The association of the MDR1 gene SNP with the tacrolimus dose requirements was evaluated in two recent studies. Goto et al. (14) found no association between 10 SNP in MDR1 gene and the tacrolimus concentration/dose ratio during the first postoperative days after liver transplantation. Conversely, MacPhee et al. (28) recently reported a weak association between the exon 26 3435C>T SNP and the dose requirement of tacrolimus 3 mo after renal transplantation. In this study, they tested only the synonymous C3435T SNP of MDR1. This SNP is a silent polymorphism that does not result in any amino acid changes. The conflicting results of these two studies and our own may be partly due to the high level of genotype characterized by the population study may limit these confounding factors. Only a prospective study may limit these confounding factors.

In summary, this study demonstrates that MDR1 gene SNP are associated with the tacrolimus requirements. Pharmacogenetic testing therefore could contribute to the individualization of drug treatment and enhance drug safety and efficacy. Genotype characterization can easily be performed before transplantation and may help to determine the initial daily dose needed after transplantation by individual patients to obtain adequate immunosuppression without increasing the risk of toxicity. It should be highlighted that these results have been obtained from a relatively small number of patients. The confirmation of the association of the MDR1 SNP with tacrolimus requirements is needed in a larger and more diverse population. Large-scale genotype-phenotype correlation trials are required to increase our knowledge of the effects of SNP on clinical outcome.

Acknowledgments
Dany Anglicheau was awarded a grant by the Groupe Coopératif d’Île-de-France. This work was supported by the Délégation à la Recherche Clinique-Hôpitaux de Paris and by the Institut National de la Santé et de la Recherche Médicale. The funding sources were not involved in study design; the collection, analysis, and interpretation of data; or the writing of this report.

References
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Table 3. Daily doses and concentration/dose ratios of tacrolimus according to the genotypes derived from the two predominant haplotypes in 81 renal transplant recipients after 1 mo of tacrolimus treatment

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Tacrolimus Requirements at 1 Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Daily Dose (mg/kg/d)</td>
</tr>
<tr>
<td>1/1</td>
<td>20</td>
<td>0.21 ± 0.07a</td>
</tr>
<tr>
<td>1/2</td>
<td>22</td>
<td>0.15 ± 0.06a</td>
</tr>
<tr>
<td>2/2</td>
<td>8</td>
<td>0.13 ± 0.07a</td>
</tr>
</tbody>
</table>

Haplotypes 1 and 2 are derived from the exon 12, 21, and 26 SNP: haplotype 1, C-G-C; haplotype 2, T-T, A-T.

*p < 0.05.