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Dopamine D3 Receptor Ser9Gly and Catechol-O-methyltransferase Val158Met Polymorphisms and Acute Pain in Sickle Cell Disease

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Abstract

Background—Pain in sickle cell disease (SCD) is characterized by episodes of acute pain, primarily responsible for acute health care utilization, and persistent chronic pain. Pain severity and frequency vary significantly among SCD patients. In this study, we investigated the possible contribution of monoamine gene polymorphisms to pain variation.

Methods—Adult subjects with SCD completed PAINReportIt®, a computerized McGill Pain Questionnaire, from which we calculated the Composite Pain Index. Utilization data were obtained from the medical record and biweekly telephone calls for 12 months. Utilization is defined as admissions to the emergency department and/or the acute care center resulting from a sickle cell pain crisis. We performed genotyping for catechol-O-methyltransferase (COMT) Val158Met (rs4680) and dopamine D3 receptor(DRD3) Ser9Gly (rs6280) polymorphisms, which were analyzed for associations with pain phenotypes.

Results—Binary logistic models revealed that DRD3 Ser9Gly heterozygote patients were more likely not to have an acute pain crisis (odds ratio [OR] [95% confidence interval (CI)], 4.37 [1.39, 22.89]; p=0.020), which remained so when demographic variables were considered (OR [95% CI], 4.53 [1.41, 28.58]; p=0.016). COMT Val158Met Met allele showed lower probability for zero utilization (OR [95% CI], 0.32 [0.12, 0.83]; p=0.020) than the Val allele. In the negative binomial regression analysis, subjects with COMT Met/Met genotype had utilization incident rate ratio [95% CI] of 2.20 [1.21, 3.99] over those with Val/Val (p=0.010).

Conclusions—These exploratory findings suggest that DRD3 Ser9Gly and COMT Val158Met may contribute to pain heterogeneity in SCD, as suggested by the different rates of acute pain crisis. Specifically, SCD patients with the DRD3 homozygote genotypes, COMT 158 Met allele or Met/Met genotype are more likely to have acute care utilization, an indicator of acute pain. These results, however, will need to be further examined in future large prospective studies.

Introduction

Pain in sickle cell disease (SCD) can be seen at a very young age and throughout a patient's life.[1,2] The acute pain episode, also known as a sickle cell (SC) pain crisis, is likely caused by vaso-occlusion and is the most common reason for hospital admissions among both adult and children SCD patients.[3-5] Frequency of these painful crises varies considerably. In a study of 29,922 SCD patients, 29.4% of patients did not experience acute pain crises, whereas 16.9% had 3 or more crises per year.[3] Chronic pain is also prevalent in SCD patients.[2,6] Wilkie et al applied a multidimensional pain surveillance tool (PAINReportIt®) to simultaneously measure pain location, intensity, quality and pattern in adults with SCD during routine outpatient clinic visits.[2] The findings indicate that nearly two-thirds of the patients experienced some pain on the day of their routine clinic visit (i.e., not a crisis day),

and 80% described their pain as constant, continuous or steady. Furthermore, almost 1 in 5 patients reported severe pain, and a third of the patients were not satisfied with their level of pain at the clinic visit.[2] Smith et al[6] found that in a daily diary assessment of SC pain, 29.3% of patients reported pain nearly every day and 14% rarely had pain. Consistent with the persistent nature of SCD pain, there is also evidence that SCD pain has both nociceptive and neuropathic components.[2,7,8]

The underlying cause of individual differences in pain in SCD remains largely unknown. Because pain is also associated with an increased mortality rate in SCD subjects,[1] understanding the heterogeneity in SCD may help to design personalized medicine to not only control pain and improve the quality of life, but also increase survival in these patients. The aim of the current study was to test the hypothesis that genetic polymorphisms may account for at least some of the pain heterogeneity in patients with SCD. The mesolimbic monoamine system is critically important for pain and comorbidity.[9,10] Examining the relationships between the genetic variation in monoamine pathways and pain may shed light on how individual differences in pain may be affected.[10,11] In this study, we focused on 2 single nucleotide polymorphisms (SNP) in catechol-O-methyltransferase (COMT) and dopamine D3 receptor (DRD3) genes from the monoamine neurotransmitter systems.

COMT is an enzyme that transfers the methyl group of S-adenosylmethionine to 3-hydroxy groups of catecholamines such as dopamine.[12] Substitution of valine by methionine at codon 158 (Val158Met, rs4680, chromosome 22 position 19951271) leads to a 3- to 4-fold reduction in enzyme activity. As 1 of the key enzymes in the degradation of catecholamines, low COMT activity has been associated with higher sensitivity to pain.[13] Rs4680 Met/Met genotype has been reported to be associated with higher sensory and affective ratings of pain.[14] Investigators in another study examined both COMT Val158Met and DRD3 Ser9Gly (rs6280, chromosome 3 position 113890815) polymorphisms and observed that the DRD3 Gly/Gly genotype, which confers the highest activity of the receptor, was associated with higher thermal pain thresholds in fibromyalgia patients.[15] DRD3 encodes one of the five dopamine receptors that mediate the actions of endogenous dopamine. In this study, we determined the influence of these SNPs on baseline pain and acute care utilization in adults with SCD.

Methods

Subjects

The IRB of the University of Illinois at Chicago approved the study. All participants provided written informed consent. Blood or buccal swab samples were collected at the University of Illinois (UI) Hospital and Health Sciences System in Chicago. We conducted analysis on 130 subjects for whom both clinical data and genetic samples were available. A power analysis was not performed *a priori* because this was an exploratory study. The statistical outcomes from this study may be limited by the small sample size; therefore, results will need to be replicated in future studies with larger sample sizes.

Utilization

Utilization is defined as admissions to the emergency department and/or the acute care center resulting from a SC pain crisis. In this study, utilization serves as a surrogate marker for acute pain in SCD. From medical record review (for UI utilization) or biweekly telephone calls (for non-UI utilization) to the patient, we documented the number of utilizations for the subsequent 12 months after the patient completed the baseline pain assessment.

Pain Assessment

Subjects completed a multidimensional computerized pain assessment using PAINReportIt®[2,16] to record baseline pain location, intensity, quality, and pattern during a routine outpatient clinic visit. PAINReportIt® is an electronic touch screen format of the 1970 version of MPQ (McGill Pain Questionnaire)[17] that can be self-administered by the patient with little or no computer experience. For each subject, we calculated a single Composite Pain Index (CPI) score to represent the multidimensional pain experience. The CPI was calculated for each patient as previously reported for SCD patients,[16] by converting the following PAINReportIt® raw score to a proportional score on a 0-100 scale, which was then summed and averaged:[17-19] (1) the number of pain sites, (2) average of current, least and worst pain intensity in the past 24 hours, (3) pain rating index total, and (4) a pain pattern score that ranges 0-6. In 122 adults with SCD, the CPI score measured at a routine clinic visit predicted the subsequent 1-year acute care utilization for pain, supporting its validity in SCD.[16]

DNA and Genotyping

DNA from peripheral blood samples was initially extracted using a modified salting out procedure described by Miller et al. [20] and subsequently, the rest of the samples were extracted using the QuickGene-mini80 isolation device and QuickGene DNA whole blood extraction method (Autogen, Holliston, Massachusetts). Buccal samples were extracted using a modified phenol/chloroform procedure adopted from Vandenbergh et al.[21] DNA samples were aliquoted and stored at -80°C. Genotyping was performed according to previously published methods for COMT Val158Met[22] and DRD3 Ser9Gly.[15]

Statistical Analysis

A χ^2 goodness of fit test was used to determine Hardy-Weinberg equilibrium. Categorical data were analyzed by χ^2 and Fisher's exact test where appropriate. To predict whether a SCD patient will have at least 1 utilization, genotype data were analyzed using a bias reduced binary logistic regression[23] and allele data were analyzed with a binary logistic regression model. These analyses allow for binary data to be adjusted for covariates and for 2 SNPs to be analyzed together. Covariates include: sex (male and female), age (19-70 years), SC type (SCD-homozygous hemoglobin S, SCD-sickle hemoglobin C, and others), and CPI (score from 0-100). These covariates have been reported to influence pain in SCD.[2,4,16] Patients with zero utilization served as case controls compared to patients who had 1 or more utilization counts. To test the effect of the SNPs on the number of utilization counts, we simultaneously analyzed both SNPs using a negative binomial regression

model[24] with and without covariates. Incident rate ratios are provided for these analyses. The negative binomial regression model treats utilization as count data whereas the binary logistic regression tests binary data (zero utilization versus at least 1 utilization). For CPI data, one-way ANOVA was performed to compare means between genotype groups and an independent samples t-test to compare means between alleles. Statistical analyses were performed using SPSS software (Version 19; IBM, Armonk, NY) and statistical software package R.[25] As an exploratory study, we did not apply critical p-values to results, but simply provided the observed significance levels to show the strength of possible associations. The results from the study are informative to, and remain to be confirmed by, future large prospective studies.

Results

Patient demographics including SC types are presented in Table 1. The DRD3 major (Gly) and minor allele (Ser) frequencies are 67.3% (n=175) and 32.7% (n=85), respectively (Table 2). The genotype frequencies are 42.3% Gly/Gly (n=55), 50.0% Gly/Ser (n=65) and 7.7% Ser/Ser (n=10). COMT major (Val) and minor allele (Met) frequencies are 68.1% (n=177) and 31.9% (n=83), respectively, and the genotype frequencies are 45.4% Val/Val (n=59), 45.4% Val/Met (n=59), and 9.2% Met/Met (n=12). There were no significant deviations from Hardy-Weinberg equilibrium for either SNP ($p>0.05$). No association of sex with genotype or allele was found for either SNP. Genotype distribution and frequencies of the 130 SC subjects into 9 different genotypic combination are summarized in Table 3.

Among patients with zero utilization, those with the heterozygous genotype of the DRD3 Ser9Gly polymorphism comprised 76.5% (n=13), the homozygote Gly/Gly genotype 17.6% (n=3) and the homozygote Ser/Ser 5.9% (n=1) (Table 4). Frequencies in patients with 1 or more acute care utilization are Gly/Ser: 46.0% (n=52), Gly/Gly: 46.0% (n=52) and Ser/Ser: 8.0% (n=9). When homozygous individuals (Ser/Ser & Gly/Gly) were combined and analyzed against heterozygotes, as has been suggested for this SNP by previous studies,[26,27-30] we observed an odds ratio of 3.81 [1.17-12.41; 95% confidence interval {CI}]; p=0.035 (Table 4). COMT Val allele comprised 82.4% of the total alleles in subjects with zero utilization, compared with only 17.6% of total alleles for Met (OR = 2.41 [0.96, 6.07; 95% CI]; p=0.055, Table 5).

In order to examine the contribution of both SNPs on predicting utilization, we used the bias reduced binary logistic regression model to simultaneously analyze Val158Met and Ser9Gly genotypes and then the binary logistic regression model for the Val158Met and Ser9Gly alleles (Table 6). Comparing the odds of having zero utilization between patients with Gly/Ser genotype and those with Gly/Gly, we found an odds ratio of 4.37 [1.39, 22.89; 95% CI] (p=0.020). Comparing the odds of having zero utilization between Ser/Ser and those with Gly/Gly, the odds ratio was 2.81 [0.11, 21.66; 95% CI]; p=0.338). Analysis of alleles showed an OR of 2.36 [1.09, 5.12; 95% CI] (p=0.030) between each additional DRD3 Ser allele and having zero utilization. For COMT Val158Met, we found an OR of 0.32 [0.12, 0.83, 95% CI] between having zero utilization and each additional Met allele (p=0.020).

We further examined models that included age, sex, SC type, and CPI as covariates in order to exclude potential bias on utilization counts by these variables (Table 7). Consistent with the previous analysis (Table 6), DRD3 heterozygote genotype showed an increased odds of having zero utilization over the homozygotes with an OR of 4.53 [1.41, 28.58; 95% CI] (p=0.016). Similarly, this analysis showed an OR of 0.36 [0.13, 0.98; 95% CI] (p=0.044) between having zero utilization and each additional COMT Met allele, consistent with our previous analysis.

To examine the influence of the SNPs on the number of utilization counts, we further simultaneously analyzed DRD3 Ser9Gly and COMT Val158Met in a negative binomial regression model (Table 8). This model was chosen to account for overdispersion in the utilization data. There was minimal difference in utilization rates between subjects of DRD3 Ser9Gly genotype and those of Gly/Gly genotype (incident rate ratio [IRR]=1.03 [0.71, 1.48; 95% CI]; p=0.879) or between Ser/Ser genotype and Gly/Gly (IRR=0.98 [0.50, 1.93; 95% CI]; p=0.959). COMT rs4680 Met/Met genotype, however, showed a 2.20 times the utilization incident rate than the Val/Val genotype (IRR=2.20 [1.21, 3.99; 95% CI]; p=0.010). The association between each additional COMT rs4680 Met allele and utilization counts was illustrated by the IRR of 1.33 [1.01, 1.75; 95% CI] (p=0.043).

Covariate adjusted models for negative binomial regression analyses confirmed that DRD3 Ser9Gly genotype did not associate with utilization counts (IRR=0.90 [0.64, 1.28; 95% CI]; p=0.558, Table 9). The IRR between COMT Met/Met genotype and Val/Val genotype was 1.85 [1.03, 3.30; 95% CI] (p=0.038).

The CPI values did not differ among DRD3 Ser9Gly genotype groups (F(2,127)=0.177, p=0.838, one-way ANOVA) or between the 2 allele groups (t(258)=-0.361, p=0.718, independent samples t-test). No meaningful CPI difference was found between Ser9Gly homozygotes and heterozygotes (t(128)=-0.594, p=0.554, independent samples t-test). Analysis of COMT Val158Met with CPI scores found no association with genotype (F(2,127)=0.044, p= 0.957, one-way ANOVA) or allele (t(258)= -0.213, p= 0.831, independent samples t-test).

Discussion

This is the first genetic study that was specifically designed to examine the influence of monoamine candidate gene polymorphisms on pain in SCD, although a genome-wide association study of SCD disease severity was reported.[31] In this study, we examined 2 SNPs that are relevant to the endogenous monoamine system and have been associated with pain phenotypes in non-SC patients. We used an innovative pain-scoring method CPI, and utilization data, to characterize pain phenotypes in SCD. In this study, utilization serves as a surrogate marker for acute pain due to crises. CPI (taken during routine clinic visits) is a quantitative and qualitative measurement of chronic pain on non-crisis days in SCD.

The Ser9Gly polymorphism results in an amino acid substitution at codon 9 in the N-terminal domain of the dopamine D3 receptor in which the Gly variant has been observed to trigger a greater cellular cAMP response to dopamine.[32] The Gly variant also displays

greater binding affinity to dopamine[33] and increased mitogen-associated protein kinase signal duration.[32] The Gly variant has also been reported to have greater reward-related dopamine release in the ventral tegmental area.[34] It was proposed that the Gly allele would decrease tonic release and increase phasic release of dopamine,[34] leading to decreased pain sensitivity since analgesic activity is proposed to be related to the phasic release of mesolimbic dopamine.[9,35] Although the exact mechanisms are yet to be elucidated, these studies have implications for the role of DRD3 in pain and analgesia.

Having an acute pain crisis resulting in a visit to the emergency department or acute care center is a significant and devastating problem because pain crises are associated with serious complications.[36] Therefore, patients having zero utilization serve as case controls in this analysis. The probability of heterozygotes having zero utilization were nearly 4.5 times of those with Gly/Gly genotypes and this remained consistent in the covariate adjusted model. The analysis of DRD3 Ser9Gly homozygotes against heterozygotes has not been frequently made since it was first proposed 2 decades ago. Heterozygote advantage allows for the accommodation of environmental conditions[37] and has been reported for this SNP in schizophrenia,[26] though it was not replicated in a subsequent meta-analysis.[38] Frequencies of heterozygote genotypes were decreased in subjects who were more vulnerable for cocaine dependence,[27,29] Tourette syndrome,[28] and substance abuse in schizophrenia patients.[30] Indeed, in the cerebrospinal fluids of healthy volunteers Jonsson et al.[39] found that DRD3 homozygous subjects had significantly higher 5-hydroxyindoleacetic acid levels than heterozygote subjects. Since 5-hydroxyindoleacetic acid is 1 of the major monoamine metabolites, the result suggested that homozygosity was associated with increased monoaminergic activity.[27] The exact mechanism of DRD3 Ser9Gly heterozygote advantage in SCD pain will need to be further studied.

In fibromyalgia patients, Gly/Gly genotype was found to be associated with higher thermal pain threshold and greater pain tolerance.[15] We have found that subjects with Gly/Gly or Ser/Ser had increased odds of having 1 or more utilization. Also in our study, Ser allele may be associated with elevated odds of having zero utilization (OR of 2.17 and 2.36, with or without covariables, respectively). In addition to unclear mechanisms for heterozygote advantage, these 2 studies were performed in subjects with different pain conditions and ethnicity backgrounds, which may have contributed to the different findings.

Treister *et al.*[40] studied dopaminergic activity relating to variable number of tandem repeat (VNTR) polymorphisms in monoamine oxidase-A (MAOA), dopamine transporter (DAT-1) and dopamine receptor 4 genes. The study investigators concluded that low dopaminergic activity was associated with high pain sensitivity. DAT-1 influences phasic dopamine but may not influence tonic dopamine.[35] Since phasic dopamine is related to analgesia, low phasic dopamine shown in the study by Treister *et al.*[40] may contribute to higher pain sensitivity. The relationship between tonic dopamine activity and pain has been suggested based on studies with the COMT Val158Met polymorphism. Zubieta et al.[14] showed that the regional μ-opioid system's response to pain was diminished in subjects with COMT Met/Met genotype compared with heterozygotes, possibly due to chronic dopaminergic overactivity that resulted in higher sensory and affective ratings of pain as well as a more negative internal affective state. Individuals who are homozygous at the Met158 allele have lower

activity of the enzyme and an increased tonic dopamine,[35,41] leading to low phasic activity and increased pain sensitivity. These relationships have also been reported in other chronic pain disorders, insomnia and depression which are interrelated and provide support for the tonic/phasic hypothesis.[9]

The COMT polymorphism Val158Met (rs4680) was first reported in association studies with schizophrenia.[42] It has since been associated with a myriad of disorders and behaviors such as fibromyalgia, aggression and suicide.[42-45] The Val/Val genotypes have the highest activity of COMT while individuals with Met/Met are expected to have the lowest activity.[13] In addition to the Met/Met genotype associating with higher sensory and affective ratings of pain,[14] it has also been shown to associate with stronger pain-related signals in several brain structures after repeated pain stimuli,[46] and more pain on days when pain catastrophizing or pain attention was increased in fibromyalgia patients.[43] COMT Val158Met is also associated with thermal pain and pressure pain stimuli in fibromyalgia patients.[47] Furthermore, healthy subjects with Met/Met genotype reported significantly more pain compared with those with Val/Val when they were subject to pain induced to the hand by a heat probe.[48] However, a few studies have been unable to find significant associations of Val158Met with pain. A study observing the susceptibility to neuropathic pain in a Spanish population did not find an association with Val158Met.[22] An acute postoperative pain study also did not find association,[49] and another study[50] did not find an association of Val158Met with nociceptive sensitivity. However, the same study[50] and several others,[14,48,50,51] found that Val158Met was primarily associated with the temporal integration of painful stimuli. In this study, we found the Met allele was associated with a lower probability of zero utilization (i.e., more acute pain) using the binomial logistic regression model that simultaneously analyzed DRD3 Ser9Gly and COMT Val158Met together. Furthermore, the Met/Met genotype showed 2.20 times the incident rate of utilization than the Val/Val genotype in the negative binomial regression model.

DRD3 Ser9Gly heterozygosity had increased odds of having zero utilization, even after factoring other variables including age, sex, SC type, and CPI, though it remains to be elucidated how Ser9Gly heterozygosity produced this advantage in acute pain in SCD. For the COMT Val158Met polymorphism the Met allele and Met/Met genotype were associated with utilization, indicative of more acute pain rates. Neither SNP, however, appears to alter baseline pain.

Individual pain difference in SCD is expected to be complex and will likely involve a number of genetic polymorphisms in various biological systems and pathways.[52,49] We took a candidate gene approach to specifically focus on 2 key members of the monoamine neurotransmitter systems based on findings in other pain types with these SNPs. We found that 2 COMT and DRD3 polymorphisms appear to influence acute pain in SCD subjects, as indicated by the odds ratio or incident rate ratio of having utilization due to acute pain crisis. We found no relationship of these polymorphisms with CPI scores (an indicator of chronic pain). In addition to candidate gene studies, approaches such as genome-wide association studies can aid the search for additional polymorphisms influencing pain in SCD.[31] Ultimately, elucidating genetic contributions made by a full set of relevant polymorphisms will help to guide the individualized management of pain in patients with SCD.

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Table 1

Demographics (N=130)

Age	**Years**
Mean	34.78
Standard deviation	11.35
Minimum	19
Maximum	70
Sex	**n (%)**
Female	86 (66.2)
Male	44 (33.8)
Ethnicity	**n (%)**
African American	127 (97.7)
Caucasian	1 (0.8)
Hispanic	2 (1.5)
Sickle Cell (SC) Type	**n (%)**
SCD-SS	99 (76.2)
SCD-SC	15 (11.5)
Other[a]	16 (12.3)
CPI Score	
Mean	40.80
SD	13.41
Minimum	14.77
Maximum	86.50
Utilization	**n (%)**
0	17 (13.1)
1	20 (15.4)
2	21 (16.1)
3	15 (11.5)
4	10 (7.7)
5	15 (11.5)
6	4 (3.1)
7	3 (2.3)
8	5 (3.8)
9	6 (4.6)
10	1 (0.8)
11	3 (2.3)
12	1 (0.8)
15	4 (3.1)
17	2 (1.5)
19	1 (0.8)

Age	Years
23	1 (0.8)
38	1 (0.8)

CPI: Composite Pain Index. Sickle cell types: sickle cell disease-homozygous hemoglobin S (SCD-SS, sickle cell anemia); sickle cell disease-sickle hemoglobin C (SCD-SC, hemoglobin S and hemoglobin C);

[a]SCD-S β° thalassemia (n=7, 5.4%); SCD-S β^{+} thalassemia (n=8, 6.2%); SCD-S α thalassemia (n=1, 0.7%).

Table 2
Genotype and allele frequencies of DRD3 Ser9Gly and COMT Val158Met

SNP	Genotype, n (%)		Allele, n (%)	
DRD3 Ser9Gly (rs6280)	**Gly/Gly**	55 (42.3)	**Gly**	175 (67.3)
	Gly/Ser	65 (50.0)	**Ser**	85 (32.7)
	Ser/Ser	10 (7.7)		
COMT Val158Met (rs4680)	**Val/Val**	59 (45.4)	**Val**	177 (68.1)
	Val/Met	59 (45.4)	**Met**	83 (31.9)
	Met/Met	12 (9.2)		

Dopamine D3 receptor (DRD3). Catechol-O-methyltransferase (COMT). No significant deviation from Hardy-Weinberg equilibrium ($p>0.05$): DRD3 Ser9Gly ($p=0.12$), COMT Val158Met ($p=0.61$).

Table 3

Nine possible genotype combinations of DRD3 Ser9Gly and COMT Val158Met

		DRD3 Ser9Gly n (%total)			Total
		Gly/Gly	Gly/Ser	Ser/Ser	
COMT Val158Met n (%total)	Val/Val	26 (20.0%)	30 (23.1%)	3 (2.3%)	59 (45.4%)
	Val/Met	27 (20.8%)	26 (20.0%)	6 (4.6%)	59 (45.4%)
	Met/Met	2 (1.5%)	9 (6.9%)	1 (0.8%)	12 (9.2%)
Total		55 (42.3%)	65 (50.0%)	10 (7.7%)	130 (100.0%)

Dopamine D3 receptor (DRD3). Catechol-O-methyltransferase (COMT).

Table 4
Association of DRD3 Ser9Gly polymorphism with utilization

DRD3 Ser9Gly genotypes with utilization.				
Utilization	**Genotype, n (%)**			**p-value**
	Gly/Gly	**Gly/Ser**	**Ser/Ser**	
0	3 (17.6)	13 (76.5)	1 (5.9)	0.061[a]
≥1	52 (46.0)	52 (46.0)	9 (8.0)	
DRD3 Ser9Gly alleles with utilization.				
Utilization	**Allele, n (%)**		**OR(95% CI)**	**p-value**
	Gly	**Ser**		
0	19 (55.9)	15 (44.1)	0.57 (0.27, 1.18)	0.128[b]
≥1	156 (69.0)	70 (31.0)		
DRD3 Ser9Gly heterozygotes & homozygotes with utilization.				
Utilization	**Heterozygotes, n(%)**	**Homozygotes, n(%)**	**OR (95% CI)**	**p-value**
0	13 (76.5)	4 (23.5)	3.81 (1.17, 12.41)	0.035[a]
≥1	52 (46.0)	61 (54.0)		

Dopamine D3 receptor (DRD3). Odds ratio (OR). Confidence interval (CI).

[a]Fisher's exact test;

[b]2-sided chi-square test.

Table 5
Association of COMT Val158Met polymorphism with utilization

COMT Val158Met genotypes with utilization.				
Utilization Counts	**Genotype, n (%)**			**p-value**
	Val/Val	**Val/Met**	**Met/Met**	
0	11 (64.7)	6 (35.3)	0 (0)	0.171[a]
≥1	48 (42.5)	53 (46.9)	12 (10.6)	
COMT Val158Met alleles with utilization.				
Utilization Counts	**Allele, n (%)**		**OR(95% CI)**	**p-value**
	Val	**Met**		
0	28 (82.4)	6 (17.6)	2.41(0.96, 6.07)	0.055[b]
≥1	149 (65.9))	77 (34.1)		

Catechol-O-methyltransferase (COMT). Odds ratio (OR). Confidence interval (CI).

[a] Fisher's exact test;

[b] 2-sided chi-square test.

Table 6
Simultaneous analysis of Ser9Gly & Val158Met in a binary logistic model

Genotypes		Probability of Zero Utilization		
		Estimates (Std Err)	OR (95% CI)	p-value
DRD3 Ser9Gly (ref=Gly/Gly)	**Gly/Ser**	1.47 (0.63)	4.37 (1.39, 22.89)	0.020
	Ser/Ser	1.03 (1.08)	2.81 (0.11, 21.66)	0.338
COMT Val158Met (ref=Val/Val)	**Val/Met**	-0.63 (0.54)	0.53 (0.16, 1.51)	0.245
	Met/Met	-2.13 (1.53)	0.12 (0.00, 1.06)	0.164
Alleles				
DRD3 Ser9Gly (ref=Gly)	**Ser**	0.86 (0.40)	2.36 (1.09, 5.12)	0.030
COMT Val158Met (ref=Val)	**Met**	-1.15 (0.49)	0.32 (0.12, 0.83)	0.020

Standard Error (Std Err). Reference (ref). Odds ratio (OR). Confidence interval (CI). Model: Logit[Probability(Zero Utilization)]~rs4680+rs6280.

Table 7

Binary logistic regression analyses with age, sex, SC type, and CPI as covariates

Genotypes		Probability of Zero Utilization		
		Estimates (Std Err)	OR (95% CI)	p-value
DRD3 Ser9Gly (ref=Gly/Gly)	**Gly/Ser**	1.51 (0.63)	4.53 (1.41, 28.58)	0.016
	Ser/Ser	0.46 (1.12)	1.59 (0.06, 14.84)	0.678
COMT Val158Met (ref=Val/Val)	**Val/Met**	-0.48 (0.56)	0.62 (0.17, 1.85)	0.392
	Met/Met	-1.61 (1.45)	0.20 (0.00, 1.84)	0.267
Alleles				
DRD3 Ser9Gly (ref=Gly)	**Ser**	0.78 (0.42)	2.17 (0.96, 4.92)	0.063
COMT Val158Met (ref=Val)	**Met**	-1.02 (0.51)	0.36 (0.13, 0.98)	0.044

Sickle cell (SC). SC types SCD-SS, SCD-SC, and Other (refer to Table 1. legend). Composite pain index (CPI). Standard Error (Std Err). Reference (ref). Odds ratio (OR). Confidence interval (CI). We did not observe nonlinearity **between** either age or CPI and the logit.

Table 8

Simultaneous analysis of Ser9Gly & Val158Met in a negative binomial regression model

Genotypes		Incidence Rate Ratio of Utilization		
		Estimates (Std Err)	IRR (95% CI)	p-value
DRD3 Ser9Gly (ref=Gly/Gly)	**Gly/Ser**	0.03 (0.19)	1.03 (0.71, 1.48)	0.879
	Ser/Ser	-0.02 (0.35)	0.98 (0.50, 1.93)	0.959
COMT Val158Met (ref=Val/Val)	**Val/Met**	-0.08 (0.19)	0.92 (0.64, 1.33)	0.661
	Met/Met	0.79 (0.30)	2.20 (1.21, 3.99)	0.010
Alleles				
DRD3 Ser9Gly (ref=Gly)	**Ser**	0.00 (0.14)	1.00 (0.76, 1.32)	0.991
COMT Val158Met (ref=Val)	**Met**	0.28 (0.14)	1.33 (1.01, 1.75)	0.043

Standard Error (Std Err). Reference (ref). Incident rate ratio (IRR). Confidence interval (CI).

Table 9

Negative binomial regression analyses with age, sex, SC type, and CPI as covariates

Genotypes		Incidence Rate Ratio of Utilization		
		Estimates (Std Err)	IRR (95% CI)	p-value
DRD3 Ser9Gly (ref=Gly/Gly)	**Gly/Ser**	-0.10 (0.18)	0.90 (0.64, 1.28)	0.558
	Ser/Ser	0.05 (0.34)	1.05 (0.54, 2.06)	0.883
COMT Val158Met (ref=Val/Val)	**Val/Met**	-0.14 (0.18)	0.87 (0.61, 1.24)	0.445
	Met/Met	0.61 (0.30)	1.85 (1.03, 3.30)	0.038
Alleles				
DRD3 Ser9Gly (ref=Gly)	**Ser**	-0.04 (0.13)	0.96 (0.74, 1.25)	0.774
COMT Val158Met (ref=Val)	**Met**	0.17 (0.13)	1.19 (0.92, 1.54)	0.197

Sickle cell (SC). SC types SCD-SS, SCD-SC, and Other (refer to Table 1. legend). Composite pain index (CPI). Standard Error (Std Err). Reference (ref). Incident rate ratio (IRR). Confidence interval (CI).