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Association of genetic variation in *COMT* gene with pain related to sickle cell disease in patients from the walk-PHaSST study

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Background: Vaso-occlusive pain episodes (VOEs) are the hallmark of sickle cell disease (SCD), and our current understanding of disease biology, treatment, and psychological covariates does not adequately explain the variability of pain in SCD. Functional variants in catechol-*O*-methyltransferase (*COMT*) gene contribute to variability in pain perception, but their impact on pain perception in African American SCD patients is not well known.

Methods: We studied *COMT* single-nucleotide polymorphisms (SNPs) rs6269, rs4633, rs4818, rs4680, and rs165599 to determine their relationship to patient self-reported pain, the number of acute VOEs, and their impact on daily life and health care utilization in 438 hemoglobin SS patients who participated in the walk-PHaSST study.

Results: In women, two risk SNPs (rs4633 and rs165599) and the corresponding haplotype (A*T*CA*A*) were associated with increased frequency of pain-related emergency room visit.

Conclusion: *COMT* functional variants may predispose SCD patients to worse acute pain in women. The association of *COMT* variants with the intensity of self-reported acute pain warrants further genetic study of pain perception in SCD.

Keyword: ER visit, catechol-O-methyltransferase, haplotype, VOE, SNP

Introduction

Vaso-occlusive pain is the hallmark of sickle cell disease (SCD). Vaso-occlusive pain episodes (VOEs) vary in frequency, duration, and severity between and within patients.[1,2] SCD-associated organ and tissue damages, such as avascular necrosis, also contribute to pain.[1,2] Similar to other human pain conditions, interindividual pain experiences in patients with SCD vary considerably. Although factors related to disease biology, treatment, and patient psychological factors may explain a part of this variability, genetic factors may also contribute to susceptibility to painful disorders and may modulate the perception of pain by individual patients.[3]

Catechol-*O*-methyltransferase (COMT) is a key enzyme in the catabolic pathway of pain-relevant neurotransmitters such as dopamine, epinephrine, and norepinephrine and plays a significant role in the perception of pain.[4–6] The enzymatic activity of COMT is partly determined by genetic factors.[7] Functional *COMT* variants are associated with interindividual differences in clinical pain experience, pain sensitivity to experimental stimulation, and response to analgesics.[3,6] The best known genetic variant in *COMT* is a single-nucleotide polymorphism (SNP), rs4680, also known as Val158Met.[6,8] The substitution of valine (Val) for methionine (Met) at position 158 of the soluble form of COMT leads to a three- to fourfold reduction in enzyme activity.[5,7] Among

healthy subjects, homozygous Met/Met individuals showed diminished regional μ-opioid system responses to pain compared with heterozygous individuals when hypertonic saline was injected into the masseter muscles.[6] In White Americans, three major *COMT* haplotypes corresponding to high pain sensitivity (HPS), average pain sensitivity (APS), and low pain sensitivity (LPS) have been reported.[5,7] These haplotypes were based on rs4680, two additional coding SNPs (rs4633 and rs4818), and a promoter SNP (rs6269) of the *COMT* gene. In a cell model, these haplotypes are associated with up to 20-fold differences in enzyme activity with LPS and HPS having the highest and lowest activities, respectively.[5,7] Functional variations in *COMT* are associated with the risk of developing chronic pain syndromes,[9] postsurgical pain,[10] and analgesia.[4] Recently, specific effects of stress and gender on *COMT* variants and pain perception have been reported.[11]

Comprehensive analysis of *COMT* genetic variants and their role in pain experienced by patients with SCD is limited. Furthermore, the distribution of *COMT* haplotypes in African American (AA) populations is not well known. In the current study, we determined *COMT* haplotypes based on five SNPs in a large cohort of AA patients with SCD and evaluated the association between *COMT* functional variants and haplotypes and self-reported pain episodes in the year and month prior to study enrollment.

Methods

Study cohort and clinical characterization

Subjects with hemoglobin SS genotypes (HbSS) from the screening phase of the walk-PHaSST, an NHLBI-sponsored study to screen patients with SCD for pulmonary hypertension (PH) and offer participation in a randomized control study of sildenafil, were included in this study.[12,13] The Institutional Review Board of the University of Pittsburgh and that of all participating institutes of the walk-PHaSST study approved this study. Investigators from each clinical site obtained written informed consent from all the participants. Subjects were evaluated by self-reported history modeled after the National Institute of Heath Pulmonary Hypertension screening survey,[14] physical examination, laboratory screening, and transthoracic Doppler echocardiography. Exclusion criteria included current pregnancy or lactation, any stroke within the last 6 weeks, and diagnosis of pulmonary embolism within the last 3 months.

Self-reported pain phenotype

At the screening visit, patients completed a health history survey, which included a self-description of acute SCD pain and listed associated treatments. Subjects provided detailed medical history including the total number of episodes of acute pain in the month and the year before screening. Acute pain was classified as 1) mild (may or may not require pain medicine and did not prevent normal daily activity), 2) moderate (required medications and caused significant changes in everyday activities), 3) severe (patient went to the emergency room [ER] but was not admitted), and 4) extremely severe (required hospital admission).

DNA isolation and genotyping

Genomic DNA was isolated from blood using the QIAamp DNA isolation kit (Qiagen NV, Venlo, the Netherlands). Five SNPs of the *COMT* gene, ie. rs6269, rs4633, rs4818, rs4680, and rs165599, were genotyped using TaqMan platform with predesigned primer and probes and 7900 DNA analyzer (ABI, Foster City, CA, USA).

Statistical analysis

We calculated site-specific allele frequencies using genotype counting and tested for departures from Hardy–Weinberg equilibrium using goodness of fit statistics. Pairwise estimates of linkage disequilibrium (LD) were measured as D' and r^2 from the diploid data.[15] Haplotype analysis was conducted using PHASE (Version 2.1)[16] to infer haplotypes from the unphased SNPs and coding the resulting haplotypes according to the number of copies present for each participant. Genetic association of the *COMT* variants with chronic pain was analyzed for both the presence and the intensity experienced by patients. For the pain intensity analysis, only subjects who reported positive pain scores were used for the association analysis. We assessed the association between the number of severe and extremely severe pain episodes and SNP or haplotype in a General Linear Model. Analyses were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). Additionally, the gender-specific analysis was conducted. Bonferroni correction was performed for significant associations to adjust for multiple comparisons. A P-value of <0.01 was considered significant for the SNP ($0.05/5=0.01$) and the haplotype ($0.05/5=0.01$)-specific analysis.

Results

Self-reported pain and associated clinical factors

The median age of the 438 patients was 36 years (12–69 years), and 51.8% were females (Table 1). Opioids and hydroxyurea usage were listed for 32.2 and 44.3% of the subjects at enrollment. Subjects who were on opioid treatment had more ER visits (severe and/or extremely severe

Table 1 Demographics, opioid, and hydroxyurea treatment and association with pain-related ER visit in the past year (HbSS, n=438)

Characteristics	Results	Effect on the number of pain episodes[a]	
		Beta	*P*-value
Age, mean (SD) (years)	36.0 (12.5)	–0.02	0.548
Female gender	227 (51.8)	0.44	0.662
Current opioid use	141 (32.2)	**4.07**	**<0.001**
Current hydroxyurea use	194 (44.3)	1.92	0.062
Acute pain in the past month[b]			
Mild	222 (51.1)	N/A	N/A
Moderate	194 (44.5)	N/A	N/A
Severe	74 (16.9)	N/A	N/A
Extremely severe	62 (14.2)	N/A	N/A
Acute pain in the past year[b]			
Mild	283 (65.5)	N/A	N/A
Moderate	296 (68.2)	N/A	N/A
Severe	197 (45.0)	N/A	N/A
Extremely severe	232 (53.0)	N/A	N/A

Notes: Results are in n (%) unless otherwise specified. [a]From a General Linear Model with number of severe and/or extremely severe pain episodes (pain-related ER visit) in the past year as outcome. [b]The number of subjects (n) who reported greater than zero of each specific type of acute pain in the month or year before enrollment in the study. The Beta and *P*-value with significances are in bold.
Abbreviations: HbSS, hemoglobin SS genotypes; ER, emergency room; N/A, not applicable.

pain episodes) in the year before screening than subjects who were not. This association remained significant after adjusting for age and gender ($P<0.001$). Hydroxyurea usage, age, and gender appeared unrelated to the number of episodes of severe and/or extremely severe pain in the year before study enrollment.

Genotype and haplotype frequencies of *COMT* variants

The distribution of rs6269, rs4633, rs4818, rs4680, and rs165599 followed Hardy–Weinberg equilibrium ($P\geq0.01$), with minor allele frequencies of 37.4, 30.5, 19.4, 28.6, and 28.1%, respectively (Table 2). Allele frequencies for these SNPs and degrees of LD among them were similar to those from the 1000 Genomes Project (http://browser.1000genomes.org/index.html) for the African (AFR) population. Except for rs6269, the European (EUR) population had much higher minor allele frequencies compared to AFR. Higher LD was observed for rs6269, rs4633, rs4818, and rs4680 in EUR than in AFR and AA populations from this study (Figure 1). The rs165599 had lower LD with any of the other SNPs in all populations. Nine haplotypes with the frequencies of >3% accounted for 97.6% of all haplotypes in our cohort (Table 2). HT2 was unique to this AA SCD cohort, and 34.2% of the subjects carried one or two copies of this haplotype.

Gender-specific allele association with more pain-related ER visits in the past month

To determine whether the *COMT* variants are associated with acute VOEs that resulted in health care utilization, we first analyzed the association of *COMT* SNPs with the presence and frequencies of pain-related ER visit in the past month and year. Since both severe and extremely severe pain episodes are defined by an ER visit, subjects are more likely able to recall them. SNP frequencies were not significantly different between the group who did, versus did not, have pain-related ER visits in the past month or year before study enrollment. Among the subjects (n=111) who experienced at least one ER visit in the past month or year, homozygotes of rs4633 or rs165599 common alleles experienced far fewer episodes of ER visit compared to carriers of the rare alleles for each of these SNPs, in the month before screening (Table 3). A gender-stratified analysis demonstrated that these associations were only in females for both SNPs and remained significant for rs165599 after SNP and gender multiple comparison correction ($P<0.005$). Adjusting for current opioid treatment did not change these results. None of the SNPs were associated with the episodes of pain-related ER visit in the past year.

Gender-specific haplotype association with more pain-related ER visits in the past month

To determine whether the haplotypes containing the risk alleles of rs4633 and rs165599 impact the episodes of pain-related ER visit in the previous month, we analyzed the association between the five common haplotypes with >5% frequencies (HT1–HT5) and this phenotype. Carriers of HT3 (A*T*CA*A*), which contains the risk alleles of both rs4633 and rs165599, had the most episodes of pain-related ER visit compared to noncarrier subjects (3.28 vs 1.80, $P=0.0004$), and this effect was more profound in females (4.06 vs 1.82, $P=0.0001$) (Table 4). After adjusting for the current opioids treatment, these *P*-values did not change. Compared to the associations of each individual SNP, the HT3 haplotype association was much stronger, suggesting a synergistic effect of these SNPs. Therefore, we designate the HT3 as a "risk haplotype" for VOE (acute pain resulted in ER visit) in female AA SCD patients.

Discussion

We present the first comprehensive report on the association of functional *COMT* variants and haplotypes with

Table 2 Allele and genotype frequencies of *COMT* SNPs and haplotypes in subjects with HbSS genotype from the walk-PHaSST study

SNP	Genotype frequency (%)			Minor allele frequency (%)[a]		
	MM	Mm	mm	AA	AFR	EUR
rs6269 (A/G)	40.0	45.2	14.8	37.4	36.0	40.4
rs4633 (C/T)	46.2	46.5	7.3	30.5	32.7	51.7
rs4818 (C/G)	64.6	32.0	3.4	19.4	16.9	39.7
rs4680 (G/A)	49.7	43.5	6.9	28.6	30.9	51.6
rs165599 (G/A)	52.8	38.3	8.9	28.1	26.4	69.1
Haplotype (>3%)	**Diplotype frequency (%)**			**Haplotype frequency (%)[b]**	**Pain responsive haplotype in American Caucasian[c]**	
	0	1	2			
HT1 (ACCGG)	55.7	39.7	4.6	24.4	HPS	
HT2 (GCCGG)	65.8	30.8	3.4	18.8	Not reported in Caucasian	
HT3 (ATCAA)	68.7	28.8	2.5	16.9	APS	
HT4 (GCGGG)	76.7	22.8	0.5	11.9	LPS	
HT5 (ATCAG)	81.1	18.0	0.9	9.9	APS	
HT6 (ACCGA)	91.8	7.8	0.5	4.3	LPS	
HT7 (GCGGA)	91.8	7.8	0.5	4.3	HPS	
HT8 (ATCGG)	92.5	7.5	0	3.8		
HT9 (ACGGG)	93.6	6.4	0	3.2		

Notes: [a]Minor allele definition based on current study (n=438). AA population was based on current study; AFR and EUR populations were based on 246 and 379 subjects from the 1000 Genomes Project (http://www.1000genomes.org/).[24] [b]The haplotype frequency was calculated by counting each specific haplotype and calculating the percentage of it based on the total haplotype count (2×438) in the 438 subjects of this study. [c]The HPS, APS, and LPS haplotypes were based on the definition by Diatchenko et al for American Caucasian.[5]

Abbreviations: AA, African American; AFR, African; APS, average pain sensitivity; COMT, catechol-*O*-methyltransferase; EUR, European; HbSS, hemoglobin SS genotypes; HPS, high pain sensitivity; LPS, low pain sensitivity; SNPs, single-nucleotide polymorphisms.

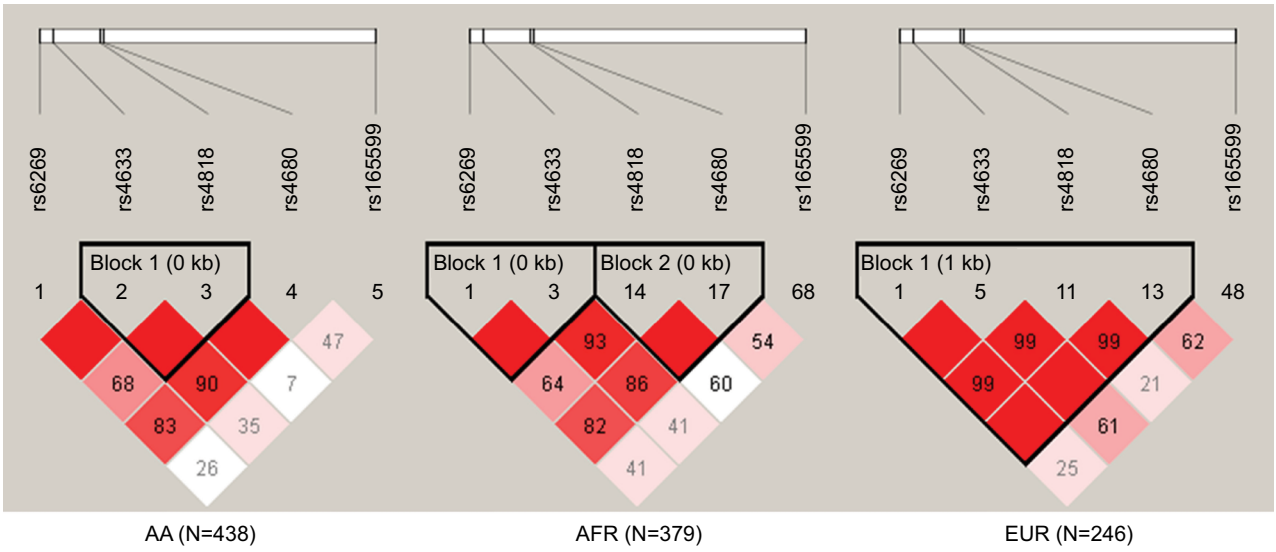

Figure 1 Haplotype structure of the *COMT* variants.

Notes: Haplotype structure was derived using Haploview. AA was based on current study. AFR and EUR were based on available data from the 1000 Genome Project (http://www.1000genomes.org/). AFR included populations of Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), and Americans of African ancestry in southwest USA (ASW). EUR included populations of America Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Toscani in Italia (TSI), Finnish in Finland (FIN), British in England and Scotland (GBR), Iberian populations in Spain (IBS).

Abbreviations: AA, African American; AFR, African; COMT, catechol-*O*-methyltransferase; EUR, European.

self-reported pain-related ER visits and health care utilizations in a large multicenter AA cohort of SCD patients. The number of episodes of pain-related ER visits was associated with current opioid use. The main finding of the effects of *COMT* variants in this SCD cohort was the association of rs4633 and rs165599 with the frequency of pain-related ER visit in the month prior to study enrollment. Additionally, we observed a strong haplotype effect on the same phenotype and identified a risk haplotype for acute pain, which resulted in health care utilization for VOEs in female patients from this AA SCD cohort.

Jhun et al[17] reported that the functional *COMT* SNP rs4680 A allele for 158Met was associated with a higher health care utilization than the Val allele in a small SCD

Table 3 Gender-specific association of *COMT* SNPs with the frequency of pain-related ER visit in the past month

SNP	N	Genotype (N) (CC/CT/TT)	Genotype mean (SE)			*P*-value*
			CC	CT	TT	
rs4633						
All	110	56/48/6	1.80 (0.17)	2.54 (0.37)	3.50 (1.38)	**0.016**
Female	66	34/29/3	1.79 (0.22)	2.86 (0.52)	5.00 (2.64)	**0.007**
Male	44	22/19/3	1.81 (0.25)	2.05 (0.49)	2.00 (0.58)	0.689
		Genotype (N) (GG/GA/AA)	**GG**	**GA**	**AA**	
rs165599						
All	111	67/35/9	1.79 (0.15)	2.86 (0.44)	3.00 (1.32)	**0.009**
Female	67	42/19/6	1.83 (0.19)	3.21 (0.65)	4.00 (1.90)	**0.004**
Male	44	25/16/3	1.72 (0.23)	2.44 (0.56)	1.00 (0)	0.713

Note: *The *P*-values were calculated based on an additive model of the specific risk alleles and *P*-values with significance are in bold.
Abbreviations: COMT, catechol-O-methyltransferase; ER, emergency room; SE, standard error, SNPs, single-nucleotide polymorphisms.

Table 4 Gender-specific association of *COMT* haplotype with the frequency of pain-related ER visit in the past month

Haplotype[a]	n	Haplotype (n) (0/1+2 copies)	Haplotype mean (SE)		*P*-value*	Risk allele (n)
			0 copy	1+2 copies		
HT1 (ACCGG)						0
All	111	57/54	2.25 (0.29)	2.20 (0.28)	0.916	
Female	67	33/34	2.61 (0.46)	2.24 (0.34)	0.517	
Male	44	24/20	1.75 (0.21)	2.15 (0.48)	0.421	
HT2 (GCCGG)						0
All	111	70/41	2.49 (0.30)	1.78 (0.15)	0.083	
Female	67	40/27	2.90 (0.45)	1.70 (0.19)	**0.034**	
Male	44	30/14	1.93 (0.34)	1.92 (0.27)	0.993	
HT3 (A***T***CA***A***)						2
All	111	79/32	1.80 (0.13)	3.28 (0.57)	**0.0004**	
Female	67	49/18	1.82 (0.17)	4.06 (0.85)	**0.0001**	
Male	44	30/14	1.77 (0.21)	2.29 (0.64)	0.327	
HT4 (GCGGG)						0
All	111	83/28	2.34 (0.25)	1.89 (0.26)	0.330	
Female	67	50/17	2.52 (0.36)	2.11(0.39)	0.540	
Male	44	33/11	2.06 (0.31)	1.55 (0.28)	0.366	
HT5 (A***T***CAG)						1
All	111	90/21	2.27 (0.22)	2.05 (0.43)	0.666	
Female	67	53/14	2.43 (0.32)	2.36 (0.63)	0.913	
Male	44	37/7	2.03 (0.29)	1.43 (0.30)	0.375	

Notes: [a]Risk alleles in each haplotype are bolded and italicized. *The *P*-values were calculated based on an additive model of the specific haplotypes and *P*-values with significance are in bold.
Abbreviations: COMT, catechol-O-methyltransferase; ER, emergency room; SE, standard error.

cohort. Our results confirm and extend their findings since the rs4680A is a part of the risk haplotype (HT3) and may also contribute to the synergistic SNP effect in the strong haplotype associations with pain-related ER visit.

Overall acute pain reflects the total burden of pain in SCD patients.[1] Since recall is extremely unreliable beyond 1 month and to avoid memory bias, we considered pain intensity in the month prior to screening to be a good marker of recent acute pain or acute exacerbation of chronic pain. We only analyzed severe and extremely severe pain as these episodes required an ER visit, which improves the validity of patient's recall. The female-specific association identified for *COMT* variants is in line with growing evidence that certain genes contribute differently to pain perception and sensitivity in men and women.[18] Moreover, *COMT* variants have demonstrated generally more profound effects in females both in mice and humans.[19] Putative mechanisms of COMT gender specificity include its regulation by estrogen and the differences in physiological responses to stressors between the genders.[19] In contrast, presentation of pain related to SCD-specific factors differed between genders in clinical studies; for instance, women with SCD were shown to have a higher rate of VOEs.[2] A recent study also revealed that *GCH1* haplotype association with pain crisis was restricted

to females with SCD.[20] Further studies are indicated to determine if independent and/or combined effects of *COMT* and *GCH1* functional alleles influence pain phenotypes in females with SCD. Because COMT variations affect both pain and analgesia, future prospective studies monitoring SCD patients before and during hospitalization are also warranted to investigate individual effects of *COMT* SNPs and haplotypes and their interactions with μ-opioid receptor (OPRM1) variants on opioid treatment outcomes.

We describe here, for the first time, the *COMT* functional haplotype distribution in AA SCD patients. The new haplotype (HT2) that was unique for AA supports population variations in the *COMT* locus. For example, Ittiwut et al[21] described a three-allele *COMT* haplotype associated with cocaine-induced paranoia in AA patients, while another *COMT* haplotype was associated with the same phenotype in White patients. This evidence may explain racial differences in erythrocyte COMT activity in AA, and White patients in that higher enzymatic activity in AA patients could be determined by functional alleles in *COMT*.[22]

The strengths of our study are that we used a large cohort of well-characterized patients who are hemoglobin SS genotype and incorporate the impact of pain on daily activities, such as work, into the definition of pain severity. However, this study had several limitations. We relied on patient self-reported ER visits and did not seek any independent verification of patient's health care utilization at various time points. We did not capture data on the moods or psychological states of these patients. This study, as other studies of pain in SCD patients, suffers from a lack of validated instruments to capture SCD pain descriptors reliably, in real time and in the patient's natural environment. We have recently developed and validated an electronic pain diary for the capture of SCD pain phenotypes in real time for use in clinical and translational research.[23] Instruments such as this may be useful in defining pain phenotype defined with accuracy and granularity in studies of genetic determinants of pain perception in SCD.

The association of *COMT* functional variants and haplotypes with patient self-reported pain phenotypes in SCD observed in this study provides the rationale for future studies to better understand the genetic regulation of pain sensitivity in AA SCD patients. Additional studies with longitudinal design and comprehensive pain assessment will help to better characterize pain phenotypes for genotype–phenotype associations. Such studies have the potential to provide insights into the molecular mechanisms associated with SCD pain and, ultimately, lead to better prevention and management of pain related to SCD.

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Author contributions

YZ, IB, and LK conceptualized the study. YC and IK performed DNA isolation and genotyping. LK and the walk-PHaSST Investigators recruited subjects and collected clinical phenotypes and blood samples. YZ and YC were involved in sample banking. YZ, MN, QZ, and RG analyzed the data. YZ,

IB, and LK drafted and finalized the article. All of the authors critically reviewed the results. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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