Research Article

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Pharmacogenomics

An alcohol dehydrogenase 7 gene polymorphism associates with both acute and chronic pain in sickle cell disease

Yavnika Kashyap[‡,1], Ying He[‡,1,2], Nilanjana Sadhu[1], Yingwei Yao[3], Diana J Wilkie[3], Robert E Molokie[1,2,4,5] & Zaijie Jim Wang*[,1,2,6,7]

[1]Department of Pharmaceutical Sciences, University of Illinois College of Pharmacy, Chicago, IL 60612, USA
[2]Comprehensive Sickle Cell Center, University of Illinois Chicago, Chicago, IL 60612, USA
[3]Department of Biobehavioral Nursing Science, University of Florida College of Nursing, Gainesville, FL 32610, USA
[4]Jesse Brown Veteran's Administration Medical Center, Chicago, IL 60612, USA
[5]Division of Hematology/Oncology, University of Illinois College of Medicine, Chicago, IL 60612, USA
[6]Department of Neurology & Rehabilitation, University of Illinois College of Medicine, Chicago, IL 60612, USA
[7]Department of Biomedical Engineering, University of Illinois Chicago College of Engineering, Chicago, IL 60607, USA
*Author for correspondence: Tel.: +1 312 355 1429; zjwang@uic.edu
‡These authors contributed equally to this work

Introduction: As the most distressing complication of sickle cell disease (SCD), pain is marked by considerable heterogenicity. In this study we explored the potential association of alcohol dehydrogenase 7 gene (*ADH7*) polymorphism rs971074 with sickle cell pain. **Methods:** We analyzed clinical phenotypes and the rs971074 single-nucleotide polymorphism in *ADH7* by MassARRAY-iPlex analysis in a cohort of SCD patients. **Results:** The synonymous rs971074 was significantly associated with both acute and chronic pain in SCD. Patients with the minor T allele(s) recorded significantly more crisis episodes and severe chronic pain symptoms. **Conclusion:** Our study has identified the rs971074 minor T allele as a genetic biomarker potentially influencing acute and chronic pain. These findings may ultimately help inform strategies to develop precision pain therapies in SCD.

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As one of the most prevalent monogenic disorders, sickle cell disease (SCD) arises from a SNP harbored in the first exon of the β-globin gene *(Hbb)*. SCD can lead to a wide range of complications that can vary in severity and frequency between individuals, including hemolytic anemia, infections, stroke, sickle retinopathy and acute chest syndrome [1]. Even within the same SCD genotype (sickle cell disease-homozygous hemoglobin S [SS], sickle cell disease-sickle β° thalassemia [Sβ°thal], sickle cell disease-sickle hemoglobin C [SC]), pain, the hallmark feature of SCD, exhibits substantial phenotype variations relating to intensity, duration and frequency, as well as the location and type of pain [2]. The highly individualized pain experience in SCD indicates the potential influences of gene polymorphisms on the pathophysiology of pain in SCD [3,4].

ADH7, which is located on chromosome 4q23, encodes class IV alcohol dehydrogenase 7 μ or σ subunit (ADH7), which participates in the first-pass metabolism of alcohol [5]. Different from the other members of *ADH* gene cluster, *ADH7* is mainly expressed in the epithelial tissues of the upper gastrointestinal tract, but not found in the liver [6]. With less efficiency in ethanol oxidation, ADH7 is more active as a retinol dehydrogenase, which potently oxidizes retinol to retinal [7]. ADH7 initiates the biosynthesis of retinoic acid, and thus functions as a crucial component in retinoid signaling [8]. Indeed, retinoic acid signaling has been implicated in gating neuropathic pain by spinal disinhibition [9]. It is plausible that ADH7 may also contribute to the development of chronic pain in SCD through the retinoic acid pathway. Notably, suboptimal plasma retinol concentrations are reported to be associated with poor clinical outcomes in SCD [10]. Serum retinol levels are inversely correlated with the degree of anemia, percentage of sickling and hospitalizations among patients with SCD [11]. Therefore, we hypothesize that ADH7 plays a key role in hemolysis, vessel occlusion and the development of the acute painful crisis.

Genome-wide studies have also identified significant associations between SNPs in *ADH7* and alcoholism, cancer, as well as substance dependence [12–14]. The rs971074 in particular has been reported to be significantly linked to

Table 1. Demographic characteristics (n = 131).

Variable	Category/statistics	Value
Sex, n (%)	Female	86 (66%)
	Male	45 (34%)
Age	Mean (SD), range	34.2 ± 11.7, 15–70
Ethnicity	African–American	131
Sickle cell type	SCD-SS	102 (78%)
	SCD-SC	15 (11%)
	SCD-Sβ+	7 (5%)
	SCD-Sβ°	7 (5%)
Composite Pain Index	Mean (SD), range	40.5 ± 13.1, 14.7–86.5
Acute care utilization	Mean (SD), range	4.4 ± 5.3, 0–38
Utilization groups	Zero (0)	19 (15%)
	Low (1–3)	57 (44%)
	High (>4)	55 (42%)

SCD: Sickle cell disease; SCD-Sβ°: Sickle cell disease-sickle β° thalassemia; SCD-Sβ+: Sickle cell disease-sickle β+ thalassemia; SCD-SC: Sickle cell disease-sickle hemoglobin C; SCD-SS: Sickle cell disease-homozygous hemoglobin S; SD: Standard deviation.

substance dependence [15] and strongly associated with upper aerodigestive tract cancers [16]. Based on these unique connections between ADH7 and SCD pain, the present study investigated the potential association of the *ADH7* polymorphism rs971074 with sickle cell pain. We examined the genotype frequencies of the rs971074 SNP and its association with acute and chronic pain in patients with SCD.

Methods

Study design

The study took place at the University of Illinois Hospital and Health Sciences System (UIHHS; IL, USA). The study was approved by the Institutional Review Board of the University of Illinois Chicago and all the participants were given a detailed explanation of the study and signed an informed consent form. Parental consent as well as child assent were obtained for participants under the age of 18 years.

Patient recruitment & clinical evaluation

Subjects with SCD who received their care from the UIHHS, the Sickle Cell Clinic and the surrounding community were recruited for the study [3,17]. SCD diagnosis included the different forms of sickle hemoglobinopathies, HbSS, HbSC and HbS beta-thalassemia. The inclusion criteria were 1) they had been diagnosed with SCD and attended UIHHS adult or pediatric Sickle Cell Clinic; 2) had sickle cell related moderate to severe pain levels (≥3 on a 0–10 scale) within 12 months before study enrollment; 3) reported at least one emergency visit or hospitalization within 2 years prior to enrollment; and 4) could speak and read English. The exclusion criteria for the study were: legally blind and individuals physically unable to complete study questionnaires.

A total of 131 African–American subjects with SCD were included in this analysis where both clinical data and genetic samples were available. The mean age of the participants was 34.2 ± 11.7 years, with 66% being female. A detailed description of demographics can be found in Table 1.

Pain assessment

Discernable pain events

The number of emergency department visits, acute care center admissions or hospitalizations over a period of 12 months after being enrolled into the study was evaluated and utilized as a marker for acute pain in SCD [17]. The events were termed as 'utilization' events and were recorded via monitoring the electronic medical record of UIHHS. The individuals were also contacted through phone every 2 weeks to document any acute visits that may have occurred at other facilities. Based on previous SCD studies, utilization groups were categorized based on the number of events as zero (0 events), low (1–3 events) or high (4–38 events) [17,18]. Figure 1 displays the distribution of subjects based on the sickle cell type and utilization groups.

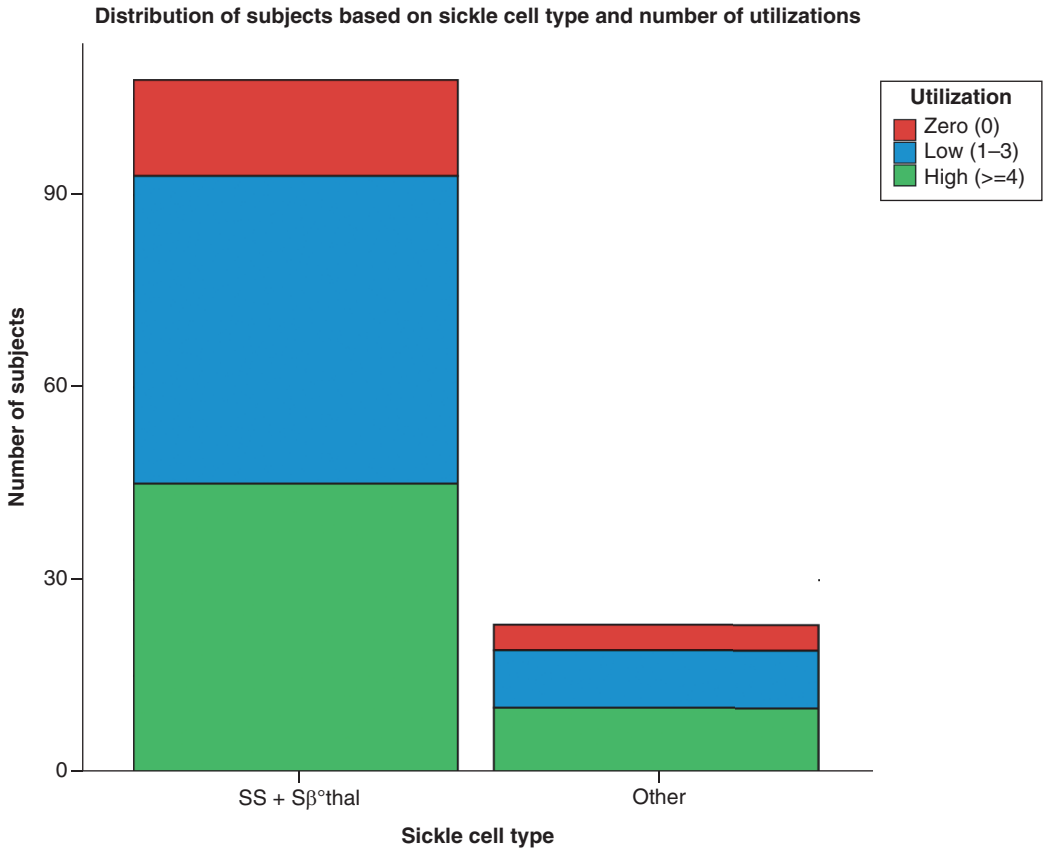

Figure 1. Distribution of subjects based on sickle cell type and utilization groups. The data are plotted as bars, with each bar representing different sickle cell disease types grouped into SS and Sβ° thal as one group and SC and Sβ+ as other. The color represents the different utilization groups, and the y-axis represents the number of subjects per group.
Sβ°: Sickle cell disease-sickle β° thalassemia; Sβ+: Sickle cell disease-sickle β+ thalassemia; SC: Sickle cell disease-sickle hemoglobin C; SS: Sickle cell disease-homozygous hemoglobin S.

Self-reported pain

An electronic version of the McGill Pain Questionnaire (MPQ) was used by the subjects to report the pain location, intensity, pattern and quality at a routine outpatient clinic visit [17,19,20]. The Composite Pain Index (CPI) was utilized as way of conceptualizing and scoring the MPQ as a patient-reported outcome [20]. A CPI score was calculated for each subject in the study based on the average pain intensity, pain pattern, pain sites and Pain Rating Index Total scores as a measure of chronic pain.

Sample acquisition & handling

The sample collection occurred at the UIHHS, which included blood and/or buccal swab samples. The samples were maintained under a cold chain (on ice) until they were to be processed for DNA extraction.

DNA extraction & genotyping

The QuickGene DNA whole-blood extraction method (AutoGen, MA, USA) was utilized for genomic DNA extraction from blood samples using QuickGene-mini80 isolation device with a modified salting out procedure [21]. The quality and quantity of extracted DNA were evaluated with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, France). The DNA extraction from the buccal samples was done using a modified phenol/chloroform procedure [22]. The SNPs were genotyped using the MassARRAY iPLEX Platform (Sequenom, CA, USA) [23]. The success rate observed for genotyping was >90%.

Table 2. *Alcohol dehydrogenase 7* polymorphism genotype and allele frequencies.

dbSNP ID	Location	Allele	Current study n (%)	Literature frequencies† n (%)		Genotype	Current study n (%)	Literature frequencies† n (%)	
				AFR	ASW			AFR	ASW
rs971074	GRCh38.p12 chr4:99420704	C	216 (82%)	1134 (86%)	102 (84%)	CC	87 (66%)	486 (74%)	41 (67%)
		T	46 (18%)	188 (14%)	20 (16%)	CT	42 (32%)	162 (25%)	20 (33%)
						TT	2 (2%)	13 (2%)	

†The 1000 Genomes population frequencies were obtained from the Ensembl Genome Browser [30]; phase III is used for comparison.
AFR: African population; ASW: African ancestry in southwest USA.

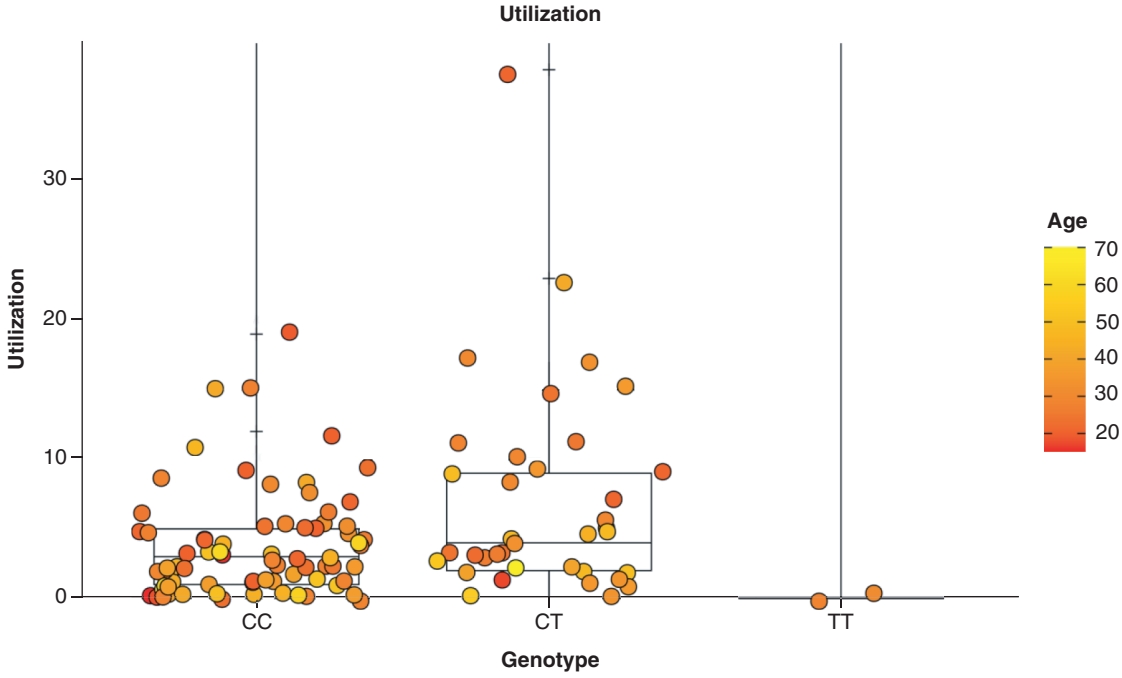

Figure 2. Distribution of subjects based on their individual utilization score and *ADH7* genotype. Each point also depicts the age of the subject. The data are plotted as boxplots and represent highest, lowest quartile and median.

Statistical analysis

The association analysis of the SNP and utilization events was done using the dominant negative binomial regression model [24,25]. The regression model was adjusted for age, sex and sickle cell type. The association analysis of SNP with the CPI scores was done using the dominant multiple linear regression model [26,27] adjusted for age, sex and sickle cell type. In addition, for subjects with sickle cell genotypes SS and Sβ°thal, a separate association analysis of the SNP with utilization and CPI scores was performed. The additive and recessive models for the SNP were not applicable due to very low minor allele frequency; therefore, only the dominant model was used. Analysis was done using Statistical Package for the Social Sciences (SPSS) and R (version 3.4.0) [28,29].

Results

Genotypic analysis of the DNA samples collected from 131 subjects, for *ADH7* SNP rs971074, demonstrated 87 patients (66%) with the CC genotype, 42 patients (32%) with the CT genotype and two patients (2%) with the TT genotype. The major and minor allele relationship for the *ADH7* polymorphisms as well as the genotypic frequencies from the current study and the expected frequencies from the 1000 Genomes Project [30] are shown in Table 2.

The mean utilization events were 3.6 ± 3.7 for participants with the CC genotype, 6.5 ± 7.3 for those with the CT genotype and none (0) for the TT genotype (Figure 2). *ADH7* rs971074 genotypes demonstrated statistically significant association with utilization events (acute pain) in the dominant regression model as observed

Table 3. Association analyses of utilization events and CPI scores with *ADH7* polymorphism.

Single-nucleotide polymorphism	Model	Incidence risk ratio (95% CI)	p-value
Utilization	Dominant (CC vs CT +TT)	1.78 (1.25–2.56)	0.001
Utilization† (SS and Sβ°thal)	Dominant (CC vs CT +TT)	1.50 (1.02–2.24)	0.03
	Model	**B (95% CI)**	**p-value**
CPI scores	Dominant (CC vs CT +TT)	6.07 (1.19–10.96)	0.015
CPI scores† (SS and Sβ°thal)	Dominant (CC vs CT +TT)	5.22 (-0.17–10.62)	0.05

Regression models are adjusted for covariates (age, sex and sickle cell type). p-values <0.05.
†SS and Sβ°thal: Sickle cell disease types (SS: Homozygous hemoglobin S, Sβ°thal: sickle β° thalassemia), regression models adjusted for covariates (age and sex).
B: Unstandardized regression coefficient; CPI: Composite Pain Index; SS: Homozygous hemoglobin S; Sβ°thal: sickle β° thalassemia.

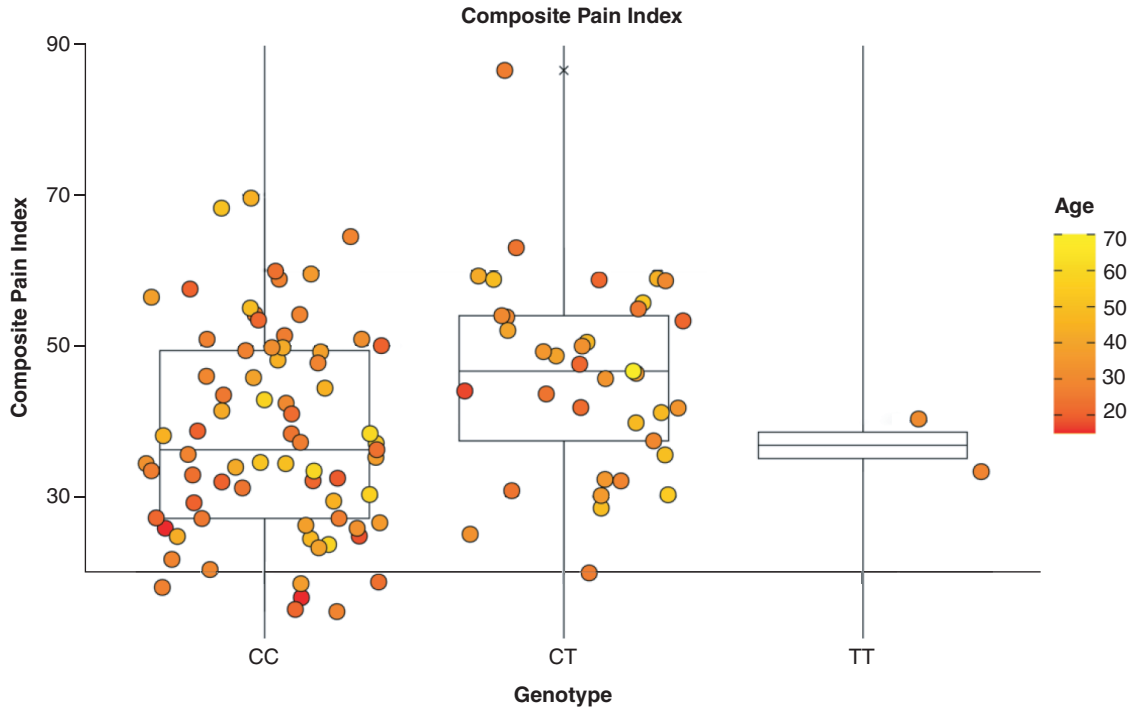

Figure 3. Distribution of subjects based on their individual Composite Pain Index score and genotype. Each point also depicts the age of the subject. The data are plotted as boxplots and represent highest, lowest quartile and median.

using negative binomial regression analysis (Table 3). The T allele was associated with a higher utilization in the dominant model (incident rate ratio [IRR] = 1.78; 95% CI: 1.25–2.56; p = 0.001; Table 3). Among subjects with for SS and Sβ°thal only, mean utilization events were 3.83 ± 3.8 for CC cohort, 5.7 ± 7.1 for CT cohort and none (0) for TT cohort. The association of T allele with higher utilization was statistically significant (IRR = 1.50; CI: 1.02 - 2.24; p = 0.03; Table 3).

In addition, we found age to be a significant predictor of utilizations (p = 0.002 for all subjects and p = 0.01 for SS and Sβ°thal only), with a decrease in the utilization events as the age increases.

The mean CPI score for the participants with the CC genotype was 38.6 ± 13.3, for the CT genotype it was 44.8 ± 13.2 and it was 36.8 ± 4.9 for participants with the TT genotype (Figure 3). The association between *ADH7* SNP rs971074 and CPI scores was also statistically significant in the dominant model (unstandardized regression coefficient [B] = 6.07; CI: 1.19–10.96; p = 0.015; Table 3). Among subjects with SS and Sβ°thal only, mean CPI scores were 38.7 ± 13.7 for participants with CC genotype, 43.4 ± 14.0 for the participants with CT genotype and 36.8 ± 3.4 for TT genotype. The association of *ADH7* SNP rs971074 with CPI was not statistically significant (unstandardized regression coefficient = 5.22; CI: -0.17–10.62; p = 0.05; Table 3) in this subset.

Discussion

SCD is a serious and lifelong autosomal recessive disorder. Patients with SCD exhibit divergent incidences of unpredictable acute painful crisis, as well as varying severities of persistent ongoing pain. The present study aimed to evaluate the genetic contribution of *ADH7* to pain heterogenicity among patients with SCD. The study included 131 self-reported African–Americans and demonstrated that the subjects with *ADH7* rs971074 minor T allele (heterozygous C/T genotype and homozygous T/T genotype) reported higher acute pain episodes and higher scores for persistent chronic pain compared with the subjects with the homozygous C/C genotype. There is an overlap in the range of utilization events, but every hospital readmission puts SCD patients at higher risks for mental health disorders, economic burden and poor quality of life [31–33].

ADH7 is a unique member of the alcohol dehydrogenase (ADH) family, which is inefficient in metabolizing ethanol while mainly catalyzes the metabolism of the longer chain aliphatic alcohols (such as retinol). The gene encoding ADH7 is at the 5′ end of the ADH gene cluster of seven ADHs. There have been several studies investigating the functional polymorphisms of *ADH7*. A synonymous SNP located in exon 6 of *ADH7*, rs971074, is found to be associated with substance dependence [14,15]. This was further corroborated by another association study conducted for heroin addiction in African–American subjects, with a finding of association between rs971074 and substance use disorder [12]. Prior to our study, no ADH7 polymorphism has been studied for its association with pain phenotype.

Synonymous SNPs can affect translation efficiency changing protein abundance [34], mRNA stability affecting the amount of mRNA available for translation, pre-mRNA splicing resulting in alternative splicing and different protein isoforms with altered function or expression patters, RNA secondary structure including mRNA folding, and stability ultimately affecting translation efficiency and protein folding [35].

ADH7 converts retinol (the major vitamin A precursor) to retinal; retinal is then synthesized to retinoic acid (the active form of vitamin A). Vitamin A deficiency (serum retinol <20 μg/dl) has been reported to be higher in cases with SCD compared with either sickle cell trait or healthy groups [11]. As a key clinical concern for SCD patients, the status of oxidative stress due to sickling and hemolysis inversely correlated with serum retinol levels. High-dose vitamin A supplementation improved hematological parameters in a randomized, double-blind pilot study in children with SCD [10]. Retinoic acid is known to play a crucial role in the maintenance of immune homeostasis during inflammatory responses and alterations in serum retinoic acid levels have been shown to be indicators of homeostatic disequilibrium [36,37]. Overall, polymorphisms in *ADH7* might alter the enzymatic activity, ultimately affecting the retinoic acid levels. Lower levels of retinoic acid might result in higher oxidative stress and imbalance in immune homeostasis, ultimately affecting pain levels.

Moreover, retinoic acid bound to its receptors regulates the expression of dopamine D2 receptors, which is a key neuromodulator [38]. As a consequence, *ADH7* indirectly regulates the development and maintenance of dopaminergic system [39]. Polymorphisms of *ADH7* thus might cause dopamine system dysfunction, which has been attributed as one of the possible mechanisms behind the association of *ADH7* rs971074 with substance dependence [15]. The dopaminergic system has been implicated for its role in nociceptive processing [40–43]. This is reinforced not only by the anatomical overlap of the regions in the brain associated with pain processing and the dopamine system, but also with considerable overlap between the cognitive and affective factors that influence the subjective experience of pain [44]. Specifically, the retinoic acid receptor, RARα, has been identified as a crucial molecular effector for neuropathic pain. Deletion of RARα in spinal cord neurons or application of an RARα antagonist in the spinal cord prevented the development of mechanical hypersensitivity in mice with spared nerve injury. Since SCD contains a neuropathic pain component, it will be interesting to investigate the linkage between the *ADH7* polymorphism and central RARα signaling in future studies.

Besides metabolism of alcohol and synthesis of retinoic acid, ADH enzymes are also involved in detoxification of reactive substances such as 4-hydroxynonenal (HNE) [45–47]. Genetic variability in the ADH sequence might affect its enzymatic function, with consequent effects on the levels of such reactive substances in the body [15]. HNE specifically has been shown to induce significant erythrocyte adhesion to endothelial cells in vascular diseases [48] and induce inflammatory pain via activation of TRPA1 receptors [49]. However, the exact involvement of ADH7 with metabolism of reactive substances warrants further investigation.

The relatively small sample size of the study and patient recruitment is a limitation. These findings need to be validated in a large prospectively designed study, along with inclusion of additional data regarding retinoic

acid/retinol levels and hematocrit levels in the patients. We also did not consider medications for the disease or pain problems that can potentially influence pain scores.

Conclusion

This is the first study reporting the association of *ADH7* SNP rs971074 with pain in SCD. We found that patients homozygous or heterozygous for the minor T allele had more recurrent episodes of pain crisis, indicating increased risk of vaso-occlusion events compared with the ones homozygous for the major C allele. In addition, rs971074 CT and TT genotypes were more frequent in patients who experience more severe chronic pain syndrome associated with SCD. These results identified a novel genetic polymorphism determinant to phenotypic variation of pain in SCD and need to be further reproduced in a larger study to develop precision pain management and personalized therapies for patients with SCD.

Summary points

- *ADH7* polymorphism rs971074 minor T allele significantly associates with higher incidence of acute pain episodes.
- The rs971074 heterozygous C/T and homozygous T/T genotypes significantly associate with more severe chronic pain.
- This is the first evidence of association of the *ADH7* polymorphism with acute crisis pain in sickle cell disease.
- This is the first evidence of association of the *ADH7* polymorphism with any chronic pain.

Author contributions

Y Kashyap and Y He were responsible for statistical analysis, interpreting data, writing, and editing the manuscript. N Sadhu was also member of the research team that collected and processed samples, performed genotyping and reviewed the statistical analysis and edited the manuscript. ZJ Wang contributed by designing the study, interpreting the data, writing, reviewing, and editing the manuscript. All other coauthors interpreted the data, reviewed and edited the manuscript.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study was approved by the Institutional Review Board of the University of Illinois Chicago and all the participants were given a detailed explanation of the study and signed an informed consent form. Parental consent as well as child assent were obtained for participants under the age of 18.

Data sharing statement

The individual participant data, that underlie the results reported in this article, after deidentification will be provided.

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