Research Article

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Pharmacogenomics

Prevalence of pain-related single nucleotide polymorphisms in patients of African origin with sickle cell disease

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Background: Prospective pain genetics research is hindered by a lack of data on the prevalence of polymorphisms in pain-relevant genes for patients with sickle cell disease (SCD). For African–Americans in general, limited information is available in public databases. **Methods:** We prioritized and examined the genotype and allele frequencies of 115 SNPs from 49 candidate pain genes in 199 adult African–Americans and pediatric patients of African origin with SCD. Analyses were performed and compared with available data from public databases. **Results:** Genotype and allele frequencies of a number of SNPs were found to be different between our cohort and those from the databases and between adult and pediatric subjects. **Conclusion:** As pain therapy is inadequate in a significant percentage of patients with SCD, candidate pain genetic studies may aid in designing precision pain medicine. We provide prevalence data as a reference for prospective genetic studies in this population.

Keywords: African–American • genotype • pain • pharmacogenomics • polymorphisms • population • sickle cell disease • SNPs

Pain is pervasive throughout the life of those with sickle cell disease (SCD) and it significantly impacts their quality of life [1–3]. SCD is characterized by both acute and chronic pain, that is highly variable in frequency and severity [4–6]. Moreover, it was found that one in three patients were not satisfied with their level of pain relief from analgesics including opioids [4]. Understanding individual differences in pain and pain relief will not only help to elucidate underlying pain mechanisms, but could have a potential to guide precision pain medicine for those with SCD [7].

There have been several genetic studies focusing on SCD, with few small studies dedicated to sickle cell pain [7–12]. Identifying genetic polymorphisms and their influence on pain phenotypes may explain some of the pain variation seen in SCD [7]. The lack of data on polymorphism frequencies for pain-relevant SNPs in SCD patients significantly hinders the proper design of prospective studies. For African–Americans, only a small number of samples with genotype and allele frequency information are found in public databases. Because of the significant genetic admixture that is found in African–Americans, many genetic studies are typically not generalizable to this population. SCD occurs in 90,000–100,000 Americans and in about 1 out of every 500 African–American births [13]. Sickle cell trait, where a person inherits one sickle hemoglobin gene and one normal hemoglobin gene, occurs in 1 in 12 African–Americans. In this study, we are able to identify and prioritize candidate genes that may contribute to pain in SCD. We have investigated 115 SNPs in a total of 49 genes that were chosen based on their relevance for pain.

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Methods

Subjects

The Institutional Review Boards of the University of Illinois at Chicago (UI) and Northwestern University (NU) approved the study. All participants gave written informed consent and provided blood or buccal swab samples. Patients were recruited during routine outpatient clinic visits (i.e., patients were not seeking immediate or urgent medical care). Eligibility criteria for patient recruitment is described in detail in another article [14]. For this study, 199 self-reported people of African background were included.

Candidate gene identification

Literature searches were performed to identify SNPs that had been associated with pain in previous studies in PubMed, using the key words: pain, polymorphism, association, genetic, pharmacogenetic and other related words including substance abuse and psychiatric disorders that are known comorbidities [15,16]. A total of 115 SNPs from 49 genes were prioritized for this exploratory study, which is not an exclusive list of all pain-related SNPs.

Genotyping

Genotyping was performed for all but four SNPs by the MassARRAY iPLEX Platform (Sequenom, CA, USA) [7]. PCR-RFLP was used to genotype the remaining four SNPs: rs1799971 [17], rs2075572 [17], rs222747 [18] and rs224534 [18], as published. Genotyping success rate was >98%.

Statistical analysis

Hardy–Weinberg equilibrium was calculated using χ^2 goodness-of-fit test with a significance level $\alpha = 10^{-3}$ [19]. The χ^2 test for independence or Fisher's exact test was used where appropriate to compare genotype or allele frequencies of different samples. Haplotypes are not analyzed for this study because of the lack of clinical relevance it would have, based on the limited number of SNPs per gene.

Results

Subject demographic data including age, gender and sickle cell genotypes are provided in Table 1 for 127 adult patients from the UI and 72 pediatric patients from NU. Combined demographics for all subjects are also provided in Table 1. The average age in the pediatric group was 14 years and 17 patients were 18 years or older. There are a disproportional number of females enrolled in this study, especially for the adult group; however, SCD itself is not known to be gender biased. Only a few studies have reported gender differences: the mortality was higher in males than females among 20–49 year old patients with SCD [20], and males were more frequently admitted for acute pain episodes whereas females had significantly longer lengths of stay in another study [21].

The genotype frequencies and allele frequencies are shown in Table 2 & Supplementary Table 1, respectively. For comparison, we also provide genotype and allele frequencies from HapMap [22] and 1000 Genomes [23] for African–Americans.

Two SNPs (rs2075507 and rs1800871) had significant deviations from Hardy–Weinberg equilibrium ($p < 0.001$) for UI and NU samples analyzed separately or combined. Two other SNPs (rs41268673 and rs121918628) were found to be monogenotypes in both UI and NU samples. These four SNPs were excluded from further statistical analyses.

We further compared our data with the literature for both allele and genotype frequencies using the χ^2 and Fisher's exact test. SNPs exhibiting significant deviation from the literature reported allele or genotype frequencies are listed in Table 3, with statistically significant p-values ($p < 0.05$) shown in bold. SNPs that are significantly different for both genotype and allele frequencies include rs1800544 in *ADRA2A*, rs1048101 in *ADRA1A*, rs167771 and rs3773679 in *DRD3*, rs10845840 in *GRIN2B*, rs13283456 in *PTGES2*, rs841718 and rs3024971 in *STAT6* and rs6357 in *TH*.

A previous study reported major opiate-related polymorphisms in an SCD cohort [24]. The survey includes genotype and allele frequency data for genes including *OPRM1* (rs1799971) and *ABCB1* (rs1045642) in an SCD cohort [24]. We found no differences between our study population and their two cohorts for these two SNPs for either genotype or allele frequencies. Between the UI and NU subjects, the genotype frequencies of 12 SNPs and allele frequencies of 12 SNPs were statistically different ($p < 0.05$) (Table 4, also noted in Supplementary Table 1). Out of these, seven SNPs differ significantly in both genotype and allele frequencies.

Discussion

Surveying 115 SNPs in 49 pain-relevant genes, we report here genotype and allele frequencies from a cohort of 199 patients with SCD who self-identified as of African origin. We found that genotype and/or allele frequencies for a number of SNPs were statistically different between our SCD cohort and literature data for African–Americans and also between our UI and NU data. The differences in the frequencies could be due to population stratification and/or sample size, among other variables. The admixture in the African–American population is about 10–20%

Table 1. Patient demographics.

		UI (n = 127)	NU (n = 72)	Total (n = 199)
Age (years)	Mean ± SD	34.9 ± 11.4	14.3 ± 4.1	27.5 ± 13.7
	Minimum	19	8	8
	Maximum	70	24	70
Gender, n (%)	Female	84 (66.1)	40 (55.6)	124 (62.3)
	Male	43 (33.9)	32 (44.4)	75 (37.7)
Sickle cell type, n (%)	SCD-SS	97 (76.4)	56 (77.8)	153 (76.9)
	SCD-SC	15 (11.8)	14 (19.4)	29 (14.6)
	SCD-β+ thal	7 (5.5)	1 (1.4)	8 (4.0)
	SCD-β° thal	7 (5.5)	1 (1.4)	8 (4.0)
	SCD-α thal	1 (0.8)	0 (0.0)	1 (0.5)

NU: Northwestern University; SC: Sickle hemoglobin C (SCD-SC, hemoglobin S and hemoglobin C); SCD: Sickle cell disease; SD: Standard deviation; SS: Homozygous hemoglobin S (SCD-SS, sickle cell anemia); thal: Thalassemia; UI: University of Illinois at Chicago.

from European genetic ancestry and the frequency differences observed between any two populations that have high admixture has been observed previously [25,26]. Allele frequency differences in admixed populations must be considered in studies performed in populations even within the same country [26]. In our study, the NU cohort includes more subjects who immigrated directly from Africa. Case control studies with admixed populations may choose to control for population stratification by methods such as genomic control or structured association to avoid spurious associations [27]. A review on association studies in structured populations has been published [28].

Several SNPs reported in this study show potential for spurious associations in an African–American population. For example, the rs1800544 SNP has been associated with various disorders in many different populations. It has been associated with tobacco smoking in Brazilians [29], antipsychotic-induced weight gain in European–Americans and Asian populations [30], and schizophrenia in a Czech population [31]. Antipsychotic-induced weight gain has been studied in an African–American population but was not significant, possibly due to small sample size [30]. These studies have accounted for genetic admixture, and it would be important to consider this for future studies that include rs1800544 to avoid any spurious associations. Our study shows that there is a significant difference in frequencies between our study population and what is reported in the literature for rs1800544.

Fifteen of our 49 genes and 49 out of 115 SNPs are related to the monoamine neurotransmitter system as a receptor, enzyme or transporter. The monoamine neurotransmitter system has previously been implicated in pain [32,33]. It is an important parameter to consider when looking for newer therapeutic strategies for sickle cell pain. As there are studies of therapies targeting the monoamine neurotransmitter system for pain [34], it will be interesting to examine the role of these polymorphisms in SCD pain. Transient receptor potential (TRP) channels, which show therapeutic promise in pain relief [35] and inflammation in SCD also play, a prominent role [6]. There also has been a study reporting frequency data on major opiate-related polymorphisms in genes including OPRM1, COMT, CYP2D6, CYP3A, UGT2B7 and ABCB1 in an SCD cohort [24]. The survey includes genotype and allele frequency data for genes including OPRM1 (rs1799971) and ABCB1 (rs1045642). We found no differences between this study cohort and our cohort for these two SNPs for either genotype or allele frequencies.

A limitation of the study is that the number and selection of SNPs included is not an exhaustive list, due to the nature of the candidate gene approach. We believe many more SNPs and genes are relevant for SCD pain as it is a complex phenotype. However, we have provided a list of pain-related SNPs in SCD to start assisting in understanding SCD pain mechanisms.

Conclusion

We have reported genotype and allele frequencies data for 115 SNPs in 49 selected pain-relevant genes from a cohort of 199 patients with SCD. These data were obtained from two different academic medical centers that serve adult and pediatric patients with SCD. These findings will facilitate prospective genetics studies for pain in SCD, including the importance of considering polymorphism frequencies in admixed populations to avoid spurious associations.

Table 2. Genotype frequencies of 115 single nucleotide polymorphisms in 49 pain-candidate genes.

Gene	dbSNP ID	Alleles		Current study			Literature frequencies†		
		Major	Minor	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)
ABCB1	1045642	C	T	87 (63.5)	41 (29.9)	9 (6.6)	35 (62.5)	19 (33.9)	2 (3.6)
	2032582	G	A	132 (75.0)	41 (23.3)	3 (1.7)	46 (80.7)	10 (17.5)	1 (1.8)
ADORA2A	3761422	C	T	54 (39.4)	68 (49.6)	15 (10.9)	20 (35.1)	26 (45.6)	11 (19.3)
ADRA1A	1048101‡	C	T	79 (58.1)	53 (39.0)	4 (2.9)	25 (43.9)	22 (38.6)	10 (17.5)
ADRA2A	521674	T	A	99 (55.9)	67 (37.9)	11 (6.2)	28 (45.9)	28 (45.9)	5 (8.2)
	602618	C	A	79 (44.6)	76 (42.9)	22 (12.4)	19 (33.3)	31 (54.4)	7 (12.3)
	1800544	G	C	79 (58.1)	53 (39.0)	4 (2.9)	20 (32.8)	30 (49.2)	11 (18.0)
	1800545	G	A	79 (57.7)	49 (35.8)	9 (6.6)	38 (62.3)	21 (34.4)	2 (3.3)
ADRB1	1801253	C	G	63 (35.4)	84 (47.2)	31 (17.4)	22 (36.1)	27 (44.3)	12 (19.7)
ADRB2	1042713	G	A	47 (26.6)	88 (49.7)	42 (23.7)	10 (17.5)	29 (50.9)	18 (31.6)
	1042717	G	A	57 (41.6)	58 (42.3)	22 (16.1)	26 (45.6)	27 (47.4)	4 (7.0)
	1432622	C	T	47 (34.3)	64 (46.7)	26 (19.0)	16 (26.2)	37 (60.7)	8 (13.1)
	1432623	T	C	49(36.0)	61 (44.9)	26 (19.1)	16 (26.2)	37 (60.7)	8 (13.1)
	2400707‡	G	A	48 (35.3)	57 (41.9)	31 (22.8)	15 (26.3)	34 (59.6)	8 (14.0)
	11958940	T	A	47 (34.3)	64 (46.7)	26 (19.0)	16 (26.2)	37 (60.7)	8 (13.1)
ANKK1	4938012	G	A	112 (62.9)	60 (33.7)	6 (3.4)	44 (72.1)	15 (24.6)	2 (3.3)
ARRB2	1045280	C	T	38 (27.7)	77 (56.2)	22 (16.1)	18 (32.1)	29 (51.8)	9 (16.1)
	2271167	A	G	51 (28.7)	97 (54.5)	30 (16.9)	19 (33.3)	29 (50.9)	9 (15.8)
AVPR1A	10877969	C	T	54 (30.7)	74 (42.0)	48 (27.3)	16 (28.1)	29 (50.9)	12 (21.1)
BDKRB2	1799722‡	C	T	68 (49.6)	60 (43.8)	9 (6.6)	26 (45.6)	23 (40.4)	8 (14.0)
BDNF	6265	G	A	165(93.2)	12 (6.8)	0 (0.0)	52 (91.2)	5 (8.8)	0 (0.0)
CACNA2D3	1851048	G	A	95(69.9)	38 (27.9)	3 (2.2)	35 (63.6)	19 (34.5)	1 (1.8)
	6777055	A	C	90 (65.7)	40 (29.2)	7 (5.1)	43 (75.4)	12 (21.1)	2 (3.5)
CHRNA4	1044396	C	T	132 (74.6)	41 (23.2)	4 (2.3)	35 (62.5)	21 (37.5)	0 (0.0)
	2236196	G	A	81 (45.8)	76 (42.9)	20 (11.3)	20 (32.8)	29 (47.5)	12 (19.7)
	4522666	G	A	134 (75.7)	39 (22.0)	4 (2.3)	36 (63.2)	21 (36.8)	0 (0.0)
	4603829	C	T	60 (33.7)	78 (43.8)	40 (22.5)	21 (36.8)	30 (52.6)	6 (10.5)

†HapMap population frequencies for African ancestry in Southwest USA (ASW) from the data release 28 Phase II + III are used for comparison. In the case ASW from HapMap was not available, 1000 Genomes population frequencies (ASW) were obtained from the Ensembl Genome Browser release 72 (Flicek *et al.* 2013).
‡SNPs showing $p < 0.05$ between University of Illinois at Chicago and Northwestern University samples by the χ^2 or Fisher's exact test.
NA: Not applicable.

Table 2. Genotype frequencies of 115 single nucleotide polymorphisms in 49 pain-candidate genes (cont.).

Gene	dbSNP ID	Alleles		Current study			Literature frequencies†		
		Major	Minor	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)
CNR1	806379	T	A	39 (28.5)	64 (46.7)	34 (24.8)	18 (29.5)	25 (41.0)	18 (29.5)
	1049353	G	A	160 (89.9)	18 (10.1)	0 (0.0)	47 (82.5)	10 (17.5)	0 (0.0)
COMT	4633	C	T	46 (33.6)	78 (56.9)	13 (9.5)	30 (52.6)	18 (31.6)	9 (15.8)
	6269‡	A	G	60 (43.8)	64 (46.7)	13 (9.5)	27 (44.3)	29 (47.5)	5 (8.2)
	737865	A	G	110 (80.3)	25 (18.2)	2 (1.5)	43 (75.4)	13 (22.8)	1 (1.8)
	4646312	T	C	98 (71.5)	36 (26.3)	3 (2.2)	42 (73.7)	15 (26.3)	0 (0.0)
DRD1	4532	T	C	109 (79.6)	25 (18.2)	3 (2.2)	44 (77.2)	12 (21.1)	1 (1.8)
DRD2	4274224	G	A	68 (50.0)	57 (41.9)	11 (8.1)	24 (42.1)	26 (45.6)	7 (12.3)
DRD3	167770‡	G	A	68 (38.6)	81 (46.0)	27 (15.3)	24 (42.1)	25 (43.9)	8 (14.0)
	167771‡	G	A	100 (56.5)	70 (39.5)	7 (4.0)	22 (38.6)	28 (49.1)	7 (12.3)
	324023‡	A	G	92 (52.0)	75 (42.4)	10 (5.6)	34 (55.7)	22 (36.1)	5 (8.2)
	324026‡	C	T	91 (51.4)	76 (42.9)	10 (5.6)	32 (56.1)	21 (36.8)	4 (7.0)
	324029‡	A	G	80 (45.2)	73 (41.2)	24 (13.6)	27 (47.4)	24 (42.1)	6 (10.5)
	905568	G	C	110 (61.8)	60 (33.7)	8 (4.5)	38 (62.3)	20 (32.8)	3 (4.9)
	1394016	A	G	132 (74.2)	41 (23.0)	5 (2.8)	34 (59.6)	21 (36.8)	2 (3.5)
	1800828	G	C	128 (71.9)	48 (27.0)	2 (1.1)	39 (63.9)	19 (31.1)	3 (4.9)
	2087017	G	A	51 (28.7)	86 (48.3)	41 (23.0)	16 (28.1)	22 (38.6)	19 (33.3)
	2134655	G	A	159 (89.3)	19 (10.7)	0 (0.0)	50 (87.7)	7 (12.3)	0 (0.0)
	2399504	G	A	151 (84.8)	25 (14.0)	2 (1.1)	44 (72.1)	16 (26.2)	1 (1.6)
	3773679	G	A	142 (79.8)	36 (20.2)	0 (0.0)	42 (68.9)	17 (27.9)	2 (3.3)
	7611535	C	T	131 (73.6)	43 (24.2)	4 (2.2)	33 (57.9)	22 (38.6)	2 (3.5)
FAAH	324419	G	A	126(70.8)	49 (27.5)	3 (1.7)	37 (64.9)	19 (33.3)	1 (1.8)
	932816	G	A	140 (78.7)	37 (20.8)	1 (0.6)	45 (73.8)	14 (23.0)	2 (3.3)
	2295632	A	C	90 (50.6)	73 (41.0)	15 (8.4)	21 (34.4)	30 (49.2)	10 (16.4)
	4141964	A	G	93 (52.2)	69 (38.8)	16 (9.0)	21 (36.8)	29 (50.9)	7 (12.3)

†HapMap population frequencies for African ancestry in Southwest USA (ASW) from the data release 28 Phase II + III are used for comparison. In the case ASW from HapMap was not available, 1000 Genomes population frequencies (ASW) were obtained from the Ensembl Genome Browser release 72 (Flicek *et al.* 2013).
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GCH1	752688	C	T	117 (65.7)	54 (30.3)	7 (3.9)	36 (59.0)	23 (37.7)	2 (3.3)
	3783641	T	A	63 (46.0)	57 (41.6)	17 (12.4)	30 (49.2)	23 (37.7)	8 (13.1)
	4411417	T	C	116 (65.5)	55 (31.1)	6 (3.4)	36 (59.0)	23 (37.7)	2 (3.3)
	8007267	A	G	53 (38.7)	63 (46.0)	21 (15.3)	19 (33.3)	28 (49.1)	10 (17.5)
GRIK3	6691840	C	A	41 (29.9)	70 (51.1)	26 (19.0)	21 (36.8)	25 (43.9)	11 (19.3)
GRIN2B	2284411	C	T	85 (47.8)	72 (40.4)	21 (11.8)	26 (45.6)	28 (49.1)	3 (5.3)
	10845840	T	C	55 (30.9)	84 (47.2)	39 (21.9)	12 (21.1)	23 (40.4)	22 (38.6)
HBS1L-MYB	4895441	A	G	142 (79.8)	35 (19.7)	1 (0.6)	41 (71.9)	15 (26.3)	1 (1.8)
	9399137	T	C	157 (88.7)	20 (11.3)	0 (0.0)	48 (84.2)	9 (15.8)	0 (0.0)
	28384513	A	C	111 (62.7)	58 (32.8)	8 (4.5)	36 (59)	23 (37.7)	2 (3.3)
HTR2A	6313	C	T	53 (39.0)	68 (50.0)	15 (11.0)	17 (30.4)	28 (50.0)	11 (19.6)
HTR3B	7943062	G	A	96(70.1)	39 (28.5)	2 (1.5)	37 (66.1)	18 (32.1)	1 (1.8)
	11214763	G	A	96(70.1)	40 (29.2)	1 (0.7)	40 (65.6)	19 (31.1)	2 (3.3)
HTR3E	4912522	G	C	85 (62.1)	48 (35.0)	4 (2.9)	31 (54.4)	24 (42.1)	2 (3.5)
	7432211	T	C	62 (45.3)	57 (41.6)	18 (13.1)	26 (45.6)	28 (49.1)	3 (5.3)
IL10	1800896	A	G	64 (46.7)	60 (43.8)	13 (9.5)	25 (43.9)	24 (42.1)	8 (14.0)
IL13	20541	C	T	98 (71.5)	38 (27.7)	1 (0.7)	35 (61.4)	21 (36.8)	1 (1.8)
IL16	4778889	T	C	73 (41.0)	78 (43.8)	27 (15.2)	19 (33.3)	33 (57.9)	5 (8.8)
IL1A	1800587	C	T	62 (34.8)	88 (49.4)	28 (15.7)	20 (35.7)	33 (58.9)	3 (5.4)
IL1B	16944	A	G	42 (30.7)	69 (50.4)	26 (19.0)	20 (35.1)	26 (45.6)	11 (19.3)
	1143634	C	T	136 (76.8)	40 (22.6)	1 (0.6)	45 (78.9)	12 (21.1)	0 (0.0)
IL1RN	2234677	G	A	150 (84.3)	27 (15.2)	1 (0.6)	52 (85.2)	9 (14.8)	0 (0.0)
IL6	1800796	G	C	113 (82.5)	24 (17.5)	0 (0.0)	51 (83.6)	10 (16.4)	0 (0.0)
KCNS1	734784	G	A	54 (30.5)	86 (48.6)	37 (20.9)	21 (36.8)	29 (50.9)	7 (12.3)
MTHFR	1801133	C	T	115 (83.9)	22 (16.1)	0 (0.0)	47 (82.5)	6 (10.5)	4 (7.0)

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		Major	Minor	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)
OPRD1	569356	T	C	160 (90.4)	17 (9.6)	0 (0.0)	54 (88.5)	7 (11.5)	0 (0.0)
	1042114	T	G	161 (90.4)	16 (9.0)	1 (0.6)	52 (91.2)	5 (8.8)	0 (0.0)
	2234918	C	T	75 (42.4)	86 (48.6)	16 (9.0)	27 (44.3)	28 (45.9)	6 (9.8)
OPRM1	1799971	A	G	191 (96.0)	8 (4.0)	0 (0.0)	53 (93.0)	4 (7.0)	0 (0.0)
	2075572	G	C	56 (28.1)	108 (54.3)	35 (17.6)	13 (21.3)	33 (54.1)	15 (24.6)
	6912029	G	T	106 (77.9)	29 (21.3)	1 (0.7)	49 (86.0)	8 (14.0)	0 (0.0)
P2RX7	2230912	A	G	160 (89.9)	17 (9.6)	1 (0.6)	54 (94.7)	3 (5.3)	0 (0.0)
PTGES2	13283456	C	T	175 (98.3)	3 (1.7)	0 (0.0)	52 (91.2)	5 (8.8)	0 (0.0)
RET	1799939	G	A	101 (73.7)	35 (25.5)	1 (0.7)	45 (81.8)	9 (16.4)	1 (1.8)
SCN1A	2298771	A	G	101 (73.7)	32 (23.4)	4 (2.9)	43 (70.5)	16 (26.2)	2 (3.3)
	121918628	C	A	178 (100.0)	0 (0.0)	0 (0.0)	NA	NA	NA
SCN9A	6746030	G	A	150 (84.7)	27 (15.3)	0 (0.0)	42 (73.7)	15 (26.3)	0 (0.0)
	41268673	G	T	178 (100.0)	0 (0.0)	0 (0.0)	59 (96.7)	2 (3.3)	0 (0.0)
SLC6A2	36020	G	A	160 (89.9)	18 (10.1)	0 (0.0)	50 (87.7)	6 (10.5)	1 (1.8)
	36029	T	C	86 (48.3)	79 (44.4)	13 (7.3)	31 (50.8)	22 (36.1)	8 (13.1)
	40147	C	T	107 (60.5)	62 (35.0)	8 (4.5)	30 (52.6)	20 (35.1)	7 (12.3)
	187715	A	G	172 (96.6)	6 (3.4)	0 (0.0)	55 (96.5)	2 (3.5)	0 (0.0)
SLC6A4	3813034‡	A	C	74 (54.0)	55 (40.1)	8 (5.8)	32 (56.1)	24 (42.1)	1 (1.8)
STAT6	167769	C	T	105 (76.6)	27 (19.7)	5 (3.6)	41 (71.9)	14 (24.6)	2 (3.5)
	841718‡	C	T	69 (50.4)	54 (39.4)	14 (10.2)	16 (28.1)	34 (59.6)	7 (12.3)
	3024971	A	C	123 (90.4)	13 (9.6)	0 (0.0)	45 (78.9)	12 (21.1)	0 (0.0)
TH	6356	G	A	99 (72.3)	34 (24.8)	4 (2.9)	43 (75.4)	13 (22.8)	1 (1.8)
	6357	G	A	146 (82.0)	32 (18.0)	0 (0.0)	44 (72.1)	14 (23.0)	3 (4.9)
	2070762	T	C	109 (61.6)	60 (33.9)	8 (4.5)	33 (58.9)	20 (35.7)	3 (5.4)
	10770141	A	G	56 (31.6)	75 (42.4)	46 (26)	22 (38.6)	24 (42.1)	11 (19.3)

†HapMap population frequencies for African ancestry in Southwest USA (ASW) from the data release 28 Phase II + III are used for comparison. In the case ASW from HapMap was not available, 1000 Genomes population frequencies (ASW) were obtained from the Ensembl Genome Browser release 72 (Flicek *et al.* 2013).
‡SNPs showing $p < 0.05$ between University of Illinois at Chicago and Northwestern University samples by the χ^2 or Fisher's exact test.
NA: Not applicable.

Table 2. Genotype frequencies of 115 single nucleotide polymorphisms in 49 pain-candidate genes (cont.).

Gene	dbSNP ID	Alleles		Current study			Literature frequencies†		
		Major	Minor	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)
TRPA1	920829	G	A	86 (48.3)	80 (44.9)	12 (6.7)	28 (49.1)	25 (43.9)	4 (7.0)
	1025928	C	T	75 (42.1)	81 (45.5)	22 (12.4)	24 (42.1)	26 (45.6)	7 (12.3)
	1443952	G	A	73 (41.0)	76 (42.7)	29 (16.3)	30 (52.6)	21 (36.8)	6 (10.5)
	3735942	G	A	70 (39.3)	74 (41.6)	34 (19.1)	29 (50.9)	22 (38.6)	6 (10.5)
	13255063	T	A	145 (81.5)	33 (18.5)	0 (0.0)	46 (80.7)	9 (15.8)	2 (3.5)
	3735943	G	A	49 (27.7)	80 (45.2)	48 (27.1)	20 (35.1)	24 (42.1)	13 (22.8)
	13279503	G	C	119 (66.9)	54 (30.3)	5 (2.8)	37 (60.7)	22 (36.1)	2 (3.3)
TRPV1	222747	C	G	154 (77.4)	40 (20.1)	5 (2.5)	46 (75.4)	14 (23.0)	1 (1.6)
	224534	C	T	171 (85.9)	26 (13.1)	2 (1.0)	51 (89.5)	6 (10.5)	0 (0.0)
	8065080	T	C	102 (74.5)	34 (24.8)	1 (0.7)	39 (69.6)	17 (30.4)	0 (0.0)
WWC1	17070145‡	T	C	56 (31.5)	85 (47.8)	37 (20.8)	16 (28.1)	31 (54.4)	10 (17.5)

†HapMap population frequencies for African ancestry in Southwest USA (ASW) from the data release 28 Phase II + III are used for comparison. In the case ASW from HapMap was not available, 1000 Genomes population frequencies (ASW) were obtained from the Ensembl Genome Browser release 72 (Flicek *et al.* 2013).
‡SNPs showing $p < 0.05$ between University of Illinois at Chicago and Northwestern University samples by the χ^2 or Fisher's exact test.
NA: Not applicable.

Table 3. SNPs differ in genotype and/or allele frequencies between the current study and those from literature.

Gene	dbSNP ID	Major alleles	Minor alleles	Genotype comparisons p-value	Allele comparisons p-value
ADRA1A	1048101	C	T	**0.002**	**0.003**
ADRA2A	1800544	G	C	**0.000**[†]	**0.000**[‡]
CHRNA4	2236196	G	A	0.112	**0.034**
COMT	4633	C	T	**0.006**	0.233
DRD3	167771	G	A	**0.014**	**0.006**
	2399504	G	A	0.065	**0.034**
	3773679	G	A	**0.028**	**0.037**
	7611535	C	T	0.071	**0.033**
FAAH	2295632	A	C	0.050	**0.014**
GRIN2B	10845840	T	C	**0.038**	**0.014**
MTHFR	1801133	C	T	**0.010**	0.189
PTGES2	13283456	C	T	**0.022**	**0.011**
SCN9A	41268673	G	T	0.064	**0.015**
STAT6	841718	C	T	**0.015**	**0.021**
	3024971	A	C	**0.036**	**0.036**
TH	6357	G	A	**0.012**	**0.023**

$p < 0.05$ is indicated in bold.
[†]$p = 0.000092$.
[‡]$p = 0.000042$.

Future perspective

Sickle cell pain is the most common and debilitating symptom in SCD that is highly variable in frequency and severity. Little is known about the underlying causes, variability and proper therapy of pain in SCD [36]. This study opens an avenue to study pain genetic mechanisms and consider precision medicine strategies for SCD pain. Genetic polymorphisms in the monoamine neurotransmitter system, TRP channels and other pain-relevant systems remain potentially fruitful areas to focus pain genetics studies. Our study has provided polymorphism prevalence data that are essential to properly design future prospective studies.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/PGS.15.126

Disclaimer

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Table 4. SNPs with significantly different genotype and/or allele frequencies between University of Illinois at Chicago and Northwestern University samples.

Gene	dbSNP ID	Major	Minor alleles	UI frequencies			NU frequencies			Genotype comparisons p-value	Allele comparisons p-value
				Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)	Homozygote (major allele), n (%)	Heterozygote, n (%)	Homozygote (minor allele), n (%)		
ADRA1A	1048101	C	T	43 (67.2)	21 (32.8)	0 (0.0)	36 (50.0)	32 (44.4)	4 (5.6)	0.040†	0.025†
ADRB2	1432622	C	T	25 (38.5)	33 (50.8)	7 (10.7)	22 (30.6)	31 (43.1)	19 (26.4)	0.066	0.049†
	1432623	T	C	27 (42.2)	30 (46.9)	7 (10.9)	22 (30.6)	31 (43.1)	19 (26.4)	0.060	0.024†
	2400707	G	A	26 (40.0)	31 (47.7)	8 (12.3)	22 (31.0)	26 (36.6)	23 (32.4)	0.020†	0.016†
BDKRB2	1799722	C	T	24 (36.9)	39 (60.0)	2 (3.1)	44 (61.1)	21 (29.2)	7 (9.7)	0.001†	0.108
COMT	6269	A	G	20 (30.8)	36 (55.4)	9 (13.8)	40 (55.6)	28 (38.9)	4 (5.6)	0.010†	0.004†
DRD1	4532	T	C	48 (73.8)	14 (21.5)	3 (4.6)	61 (84.7)	11 (15.3)	0 (0.0)	0.102	0.043†
DRD3	167770	G	A	32 (30.5)	57 (54.3)	16 (15.2)	36 (50.7)	24 (33.8)	11 (15.5)	0.015†	0.059
	167771	G	A	52 (49.5)	50 (47.6)	3 (2.9)	48 (66.7)	20 (27.8)	4 (5.6)	0.027†	0.117
	324023	A	G	45 (42.9)	55 (52.4)	5 (4.8)	47 (65.3)	20 (27.8)	5 (6.9)	0.005†	0.035†
	324026	C	T	44 (41.9)	56 (53.3)	5 (4.8)	47 (65.3)	20 (27.8)	5 (6.9)	0.003†	0.028†
	324029	A	G	38 (36.2)	52 (49.5)	15 (14.3)	42 (58.3)	21 (29.2)	9 (12.5)	0.011†	0.020†
FAAH	932816	G	A	89 (84.0)	17 (16.0)	0 (0.0)	51 (70.8)	20 (27.8)	1 (1.4)	0.072	0.031†
SLC6A4	3813034	A	C	41 (63.1)	18 (27.7)	6 (9.2)	33 (45.8)	37 (51.4)	2 (2.8)	0.011†	0.309
STAT6	841718	C	T	25 (38.5)	31 (47.7)	9 (13.8)	44 (61.1)	23 (31.9)	5 (6.9)	0.027†	0.008†
WWC1	17070145	T	C	25 (23.6)	57 (53.8)	24 (22.6)	31 (43.1)	28 (38.9)	13 (18.1)	0.022†	0.025†

†Statistically significant SNPs showing genotype or allele frequency differences between UI and NU samples.
NU: Northwestern University; UI: University of Illinois at Chicago.

Executive summary

- Only a small number of African–American samples with genotype and allele frequency information are found in public databases.
- The data are even rarer for pain-relevant SNPs in sickle cell disease patients.
- We identified 115 SNPs in a total of 49 genes based on their relevance to sickle cell or nonsickle cell pain.
- Several SNP frequencies were statistically different between our cohort and data derived from the literature, including nine SNPs that differ in both genotype and allele frequencies.
- The genotype frequencies of 12 SNPs and allele frequencies of 12 SNPs were statistically different ($p < 0.05$) between the University of Illinois at Chicago adult and Northwestern Univeristy pediatric samples.
- Allele frequency differences in admixed populations must be considered in studies to avoid spurious associations.
- Several SNPs reported in this study show potential for spurious associations in an African–American population.
- Findings reported here can help design large prospective pain genetic studies that may ultimately lead to precision therapies for pain in sickle cell disease.

References

1 Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 376(9757), 2018–2031 (2010).

2 Platt OS, Thorington BD, Brambilla DJ *et al.* Pain in sickle cell disease. Rates and risk factors. *N. Engl. J. Med.* 325(1), 11–16 (1991).

3 Kanter J, Kruse-Jarres R. Management of sickle cell disease from childhood through adulthood. *Blood Rev.* 27(6), 279–287 (2013).

4 Wilkie DJ, Molokie R, Boyd-Seal D *et al.* Patient-reported outcomes: descriptors of nociceptive and neuropathic pain and barriers to effective pain management in adult outpatients with sickle cell disease. *J. Natl Med. Assoc.* 102(1), 18–27 (2010).

5 Smith WR, Scherer M. Sickle-cell pain: advances in epidemiology and etiology. *Hematology Am. Soc. Hematol. Educ. Program* 2010, 409–415 (2010).

6 Ballas SK, Gupta K, Adams-Graves P. Sickle cell pain: a critical reappraisal. *Blood* 120(18), 3647–3656 (2012).

7 Jhun E, He Y, Yao Y *et al.* Dopamine D3 receptor Ser9Gly and catechol-O-methyltransferase Val158Met polymorphisms and acute pain in sickle cell disease. *Anesth. Analg.* 119(5), 1201–1207 (2014).

8 Shord SS, Cavallari LH, Gao W *et al.* The pharmacokinetics of codeine and its metabolites in Blacks with sickle cell disease. *Eur. J. Clin. Pharmacol.* 65(7), 651–658 (2009).

9 Lettre G, Sankaran VG, Bezerra MA *et al.* DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc. Natl Acad. Sci. USA* 105(33), 11869–11874 (2008).

10 Mendonca TF, Oliveira MC, Vasconcelos LR *et al.* Association of variant alleles of *MBL2* gene with vasoocclusive crisis in children with sickle cell anemia. *Blood Cells Mol. Dis.* 44(4), 224–228 (2010).

11 Belfer I, Youngblood V, Darbari DS *et al.* A GCH1 haplotype confers sex-specific susceptibility to pain crises and altered endothelial function in adults with sickle cell anemia. *Am. J. Hematol.* 89(2), 187–193 (2014).

12 Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. *Am. J. Hematol.* 87(8), 795–803 (2012).

13 National Heart, Lung, And Blood Institute. Disease and conditions index. Sickle cell anemia: who is at risk? www.nhlbi.nih.gov

14 Ezenwa MO, Molokie RE, Wang ZJP *et al.* Outpatient pain predicts subsequent one-year acute health care utilization among adults with sickle cell disease. *J. Pain Symptom Manage.* 48(1), 65–74 (2014).

15 Krashin D, Murinova N, Ballantyne J. Management of pain with comorbid substance abuse. *Curr. Psychiatry Rep.* 14(5), 462–468 (2012).

16 Sharp J, Keefe B. Psychiatry in chronic pain: a review and update. *Curr. Psychiatry Rep.* 7(3), 213–219 (2005).

17 Bergen AW, Kokoszka J, Peterson R *et al.* Mu opioid receptor gene variants: lack of association with alcohol dependence. *Mol. Psychiatry* 2(6), 490–494 (1997).

18 Van Esch AA, Lamberts MP, Te Morsche RH *et al.* Polymorphisms in gene encoding TRPV1-receptor involved in pain perception are unrelated to chronic pancreatitis. *BMC Gastroenterol.* 9 97 (2009).

19 Balding DJ. A tutorial on statistical methods for population association studies. *Nat. Rev. Genet.* 7(10), 781–791 (2006).

20 Shankar SM, Arbogast PG, Mitchel E *et al.* Medical care utilization and mortality in sickle cell disease: a population-based study. *Am. J. Hematol.* 80(4), 262–270 (2005).

21 Ballas SK. Pain management of sickle cell disease. *Hematol. Oncol. Clin. North Am.* 19(5), 785–802 (2005).

22 Hapmap. International HapMap Project. HapMap data release 28 Phase II + III August 10 on NCBI B36 assembly dbSNP b126 (2010). http://hapmap.ncbi.nlm.nih.gov/

23 Flicek P, Ahmed I, Amode MR *et al.* Ensembl 2013. *Nucleic Acids Res.* 41, D48–D55 (2013).

24 Joly P, Gagnieu MC, Bardel C *et al.* Genotypic screening of the main opiate-related polymorphisms in a cohort of 139 sickle cell disease patients. *Am. J. Hematol.* 87(5), 534–536 (2012).

25 Parra EJ, Marcini A, Akey J *et al.* Estimating African–American admixture proportions by use of population-

specific alleles. *Am. J. Hum. Genet.* 63(6), 1839–1851 (1998).

26 Marsh S, King CR, Van Booven DJ *et al.* Pharmacogenomic assessment of Mexican and Peruvian populations. *Pharmacogenomics* 16(5), 441–448 (2015).

27 Price AL, Patterson NJ, Plenge RM *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* 38(8), 904–909 (2006).

28 Roeder K, Luca D. Searching for disease susceptibility variants in structured populations. *Genomics* 93(1), 1–4 (2009).

29 Prestes AP, Marques FZ, Hutz MH *et al.* Tobacco smoking and the ADRA2A C–1291G polymorphism. *J. Neural Transm.* 114(11), 1503–1506 (2007).

30 Sickert L, Muller DJ, Tiwari AK *et al.* Association of the alpha 2A adrenergic receptor –1291C/G polymorphism and antipsychotic-induced weight gain in European–Americans. *Pharmacogenomics* 10(7), 1169–1176 (2009).

31 Lochman J, Balcar VJ, Stastny F, Sery O. Preliminary evidence for association between schizophrenia and polymorphisms in the regulatory Regions of the *ADRA2A*, *DRD3* and *SNAP–25* genes. *Psychiatry Res.* 205(1–2), 7–12 (2013).

32 Delgado PL. Common pathways of depression and pain. *J. Clin. Psychiatry* 65(Suppl. 12), 16–19 (2004).

33 Wood PB. Role of central dopamine in pain and analgesia. *Expert Rev. Neurother.* 8(5), 781–797 (2008).

34 Rosenberg MB, Carroll FI, Negus SS. Effects of monoamine reuptake inhibitors in assays of acute pain-stimulated and pain-depressed behavior in rats. *J. Pain* 14(3), 246–259 (2013).

35 Brederson JD, Kym PR, Szallasi A. Targeting TRP channels for pain relief. *Eur. J. Pharmacol.* 716(1–3), 61–76 (2013).

36 Wright J, Ahmedzai SH. The management of painful crisis in sickle cell disease. *Curr. Opin. Support. Palliat. Care* 4(2), 97–106 (2010).