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IL1A rs1800587 associates with chronic noncrisis pain in sickle cell disease

Aim: Pain is prevalent in sickle cell disease (SCD) patients who display great heterogeneity in pain severity and frequency. Hypothesizing that inflammatory factors are involved in the pathogenesis of SCD pain, we focused on the *IL1A* C/T polymorphism rs1800587 that is an SNP located in a *cis*-transcriptional regulatory region. **Methods:** We genotyped *IL1A* rs1800587 and performed association studies with phenotype data obtained by a multidimensional pain assessment tool using the PAINReportIt® Questionnaire. **Results:** Each T allele was associated with a 3.9 increase in composite pain index score ($p = 0.04$) as determined by multiple linear regression. **Conclusion:** *IL1A* rs1800587 may influence chronic pain in SCD.

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Pain is prevalent and severe in patients with sickle cell disease (SCD), an inherited hematological disorder caused by substitution of a valine residue for glutamic acid in the 6th codon of the β -globin gene [1–3]. Although the genetic basis of the disease is well defined, the pathogenesis and heterogeneity of pain in SCD remains poorly understood. Episodes of vaso-occlusive crisis, which play an important role in SCD pathophysiology, result in severe acute crisis pain that is the most common reason for hospital admissions [4–6]. In addition to, and in absence of acute crisis pain, nearly half of SCD patients experience chronic pain that is refractory to currently available medications, presenting a challenging clinical problem [2,6,7]. Moreover, both acute and chronic pain are highly heterogeneous [1,2,4,7,8].

SCD patients are known to have heightened inflammation. Pro-inflammatory cytokines derived from platelets, white blood cells and endothelial cells have all been implicated

in the development and maintenance of SCD-associated acute and chronic pain [9–11]. Elevation in pro-inflammatory cytokines and reduction in anti-inflammatory cytokine levels have been reported in SCD patients during the vaso-occlusive crisis [12–14]. Among the pro-inflammatory cytokines, IL-1 is a pivotal factor controlling the inflammatory response. There are two genetically distinct molecular forms of IL-1: IL-1 α and IL-1 β , which bind to the same receptor. Extensive studies have been performed on various immune functions of IL-1 β and its participation in inflammatory and neuropathic pain [15]. IL-1 β promotes systemic inflammation through activation of cyclooxygenase-2 and also enhances the production of substance P, nitric oxide and endothelial adhesion molecules that promote pain. IL-1 β has been implicated as playing an important role in the development and maintenance of postoperative pain, nerve injury-induced pain and pain in experimen-

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tal autoimmune encephalomyelitis [16–18]. Recent publications also suggested an association between IL-1β and manifestations of SCD [19,20].

Much less is known about the involvement of IL-1α in pain and in SCD. It has been implicated in a study using cultured dorsal root neurons that IL-1α is involved in pain due to nerve injury including upregulating substance P release and altering nerve growth factor expression [21]. Another study showed increased expression of IL-1α in the spinal cord after painful joint injury and suggested that IL-1α may contribute to the generation of pain [22]. *IL1A* rs1800587 C>T is an SNP located at position -889 upstream of translation start (+1), which is within a transcriptional regulatory region. The TT genotype was reported to increase promoter activity, producing increased levels of IL-1α mRNA and IL-1α, compared with those of CC genotype [23]. A recent study found that carrying the *IL1A* T allele (CT and TT genotypes) was associ-

ated with increased pain intensity and reduced pressure pain threshold in patients with lumbar radicular pain due to symptomatic disk herniation and lower back pain [24,25]. In this study, we studied the influence of this *IL-1A* SNP on acute and chronic pain in adults with SCD.

Methods
Subjects

The Institutional Review Board of the University of Illinois at Chicago approved the study. Subjects were recruited during routine clinic visits at the University of Illinois (UI) Hospital and Health Sciences System in Chicago (IL, USA). Participants provided written informed consent before blood or buccal swab samples were collected for DNA extraction. A total of 115 subjects are included in this study for whom both clinical pain phenotype and genetic samples were available.

Assessment of chronic pain

A computerized multidimensional pain assessment tool, PAINReportIt® [2,26,27] (Nursing Consult, LLC, WA, USA), was used for pain phenotyping. Subjects completed baseline pain location, intensity, quality and pattern during a routine clinic visit (i.e., not during pain crisis or other urgent care visits to the clinic). The composite pain index (CPI) score is a value on a 0–100 scale that is calculated from these raw scores: number of pain sites; average of current, least and worst pain intensity in the past 24 h; pain rating index total; and a pain pattern score that ranges from 0 to 6 [28–30]. This score represents the multidimensional pain experience at baseline.

Assessment of acute pain

Visits or admission to the emergency department or acute care center as a result of a SCD pain crisis, denoted as utilization, was used as a surrogate marker for an acute crisis pain in SCD [31]. We documented the number of these utilizations each patient had for 12 months after completing the baseline pain assessment by reviewing medical records for utilizations made at UI or by biweekly telephone calls in the case that patients did not go to the UI for care but went to any nearest emergency department.

DNA extraction & genotyping

DNA was extracted from peripheral blood samples by a modified salting out procedure [32] or the QuickGene-mini80 isolation device and QuickGene DNA whole blood extraction method (AutoGen, MA, USA). DNA from buccal samples were extracted using a modified phenol/chloroform procedure [33]. Genotyping was performed by the MassARRAY iPLEX Platform

Table 1. Patient demographics.		
Demographic name	Description	All patients (n = 115)
Age	Mean ± SD	34.1 ± 12.3
	Minimum	15
	Maximum	70
Sex, n (%)	Female	78 (68)
	Male	37 (32)
Ethnicity, n (%)	African–American	111 (97)
	Hispanic	3 (3)
	Caucasian	1 (1)
Sickle cell type*, n (%)	SCD-SS	93 (81)
	SCD-SC	11 (10)
	SCD-Sβ ⁺	5 (4)
	SCD-Sβ ^o	5 (4)
	SCD-Sα	1 (1)
CPI	Mean ± SD	40.7 ± 13.6
	Minimum	14.8
	Maximum	86.5
Utilization	Mean ± SD	4.4 ± 5.4
	Minimum	0
	Maximum	38
Utilization groups, n (%)	Zero (0)	17 (15)
	Low (1–3)	52 (45)
	High (4–38)	46 (40)
CPI: Composite pain index; SCD-Sα: Sickle cell disease-sickle α thalassemia; SCD-Sβ ⁺ : Sickle cell disease-sickle β ⁺ thalassemia; SCD-Sβ ^o : Sickle cell disease-sickle β ^o thalassemia; SCD-SC: Sickle cell disease-sickle hemoglobin C; SCD-SS: Sickle cell disease-homozygous hemoglobin S, sickle cell anemia.		

(Sequenom, CA, USA) where a single primer extension occurred upstream of the polymorphic site and was subsequently measured by MALDI-TOF MS [34].

Statistical analysis

Hardy–Weinberg equilibrium was determined by a χ^2 goodness-of-fit test. Additive (allele effects), dominant (combined major homozygous genotype and heterozygous vs minor homozygous genotypes) and recessive (major homozygous genotypes vs combined heterozygous and combined minor homozygous) multiple linear regression models [35,36], adjusted for age, sex, ethnicity and sickle cell type, were used for analysis of statistical association of the SNP with CPI (i.e., chronic pain). These genetic models were used because the mode of inheritance is not known for this SNP and it is a powerful way of detecting the effect of the SNP on phenotype data, although we do not correct for multiple testing in this exploratory analysis [35]. Association with utilization (i.e., acute pain) was analyzed by additive, dominant and recessive negative binomial regression models [37] adjusted for the same covariates. The negative binomial regression model is used for our utilization data due to overdispersed count outcome variables [38]. In other words, the utilization variances that are found within each genotype group are higher than the mean utilization of that same genotype group. SNP effects on utilization groups (three groups: zero events, 1–3 events and 4–38 events) were also analyzed by an ordinal logistic regression model adjusted for covariates [26]. Analyses were performed on SPSS software (version 22; IBM, NY, USA).

Bioinformatics

The University of California at Santa Cruz Genome Browser (Human Feb. 2009 GRCh37/hg19 Assembly) [39] was used to obtain Encyclopedia of DNA Elements at University of California at Santa Cruz (ENCODE) data for DNase clusters [40,41,42]. ENCODE allows users to view functional elements within the human genome, including regulatory elements that were viewed for this study.

Table 2. *IL1A* rs1800587 genotype and allele frequencies.

	n	%
Genotype		
CC	40	35
CT	55	48
TT	20	17
Allele		
C	135	59
T	95	41

Results

Patient demographics for all 115 subjects are provided in **Table 1**. The mean age in this study was 34.1 years with a range of 15–70 years. The majority of our subjects enrolled are females, although we did not attempt to bias recruitment of females. Our cohort is predominantly self-reported African–Americans (97%) with three Hispanics and one Caucasian. The sickle cell genotypes that are present in our cohort include the sickle cell anemia group (SCD-SS, 80.9%), sickle hemoglobin C (SCD-SC, 9.6%) and sickle alpha and beta thalassemias (9.5%). The mean CPI was 40.7 with a wide range from 14.8 to 86.5. Number of utilization ranged from 0 to 38 within 1 year. Three groups are created from utilization rates: 0 utilization (15% of total patients), 1–3 (45%) and 4–38 (40%) based on previous studies where the distribution of acute healthcare utilization events were taken into account [26].

IL1A rs1800587 genotype and allele frequencies are provided in **Table 2**. No significant deviations from Hardy–Weinberg equilibrium were found for this SNP ($p > 0.05$). The T allele was associated with an increase of 3.85 in CPI score ($B = 3.85$ [95% CI: 0.15–7.56]; $p = 0.042$), additive multiple linear regression model (**Table 3**) and accounted for 3% of the variability in CPI (adjusted R^2). In order to determine if there are effects of this SNP on phenotype in a dominant or recessive mode, we also used dominant and recessive multiple linear regression models to analyze the data. However, these latter analyses did not yield

Table 3. Multiple linear regression model evaluating the effects of *IL1A* rs1800587 on chronic pain in sickle cell disease

Model	B (95% CI) [†]	p-value	R ²	Adj. R ^{2‡}
Additive	3.85 (0.15–7.56)	0.042	0.11	0.03
Dominant	5.66 (-1.26–12.58)	0.108	0.10	0.02
Recessive	4.62 (-0.77–10.02)	0.092	0.10	0.02

The major allele is the reference genotype in the analyses. Regression models are adjusted for age, sex, ethnicity and sickle cell type.

[†]Unstandardized regression coefficient and 95% confidence interval.

[‡]Adjusted R-square for six predictors (SNP, age, sex, ethnicity and sickle cell type).

Figure 1. Composite pain index versus rs1800587 genotypes by additive, dominant and recessive models. Middle bar: median composite pain index, top of box: 75% tile, bottom of box: 25% tile, top bar: maximum, bottom bar: minimum, outlier: >2 box lengths above the 75% tile. Boxplot representation does not account for the variance caused by covariates (age, sex, ethnicity, sickle cell type and utilization).

statistically significant findings ($p = 0.108, 0.092$, respectively).

The boxplot representation of the CPI score versus genotypes in an additive, dominant and recessive model is shown in **Figure 1**. A trend can be observed for an increased CPI score with the T allele for each graphical representation.

We also determined the association between rs1800587 and utilization, which is used as a surrogate marker for acute crisis pain [31]. There was no statistical association between utilization and rs1800587 in additive, dominant or recessive models (incident rate ratio [IRR] = 0.93, 0.93, 0.90 [95% CI: 0.70–1.23, 0.55–1.56, 0.60–1.35]; $p = 0.606, 0.774, 0.605$, respectively, **Table 4**). Rs1800587 also did not associate with utilization groups when analyzed by the ordinal logistic regression model where the groups were categorized into high, low and zero utilization based on prior studies by additive, dominant and recessive models (estimate = -0.02, 0.22, -0.18 [95% CI: -0.54–0.51, -0.75–1.20, -0.94–0.58]; $p = 0.949, 0.652, 0.637$, respectively, **Table 4**) [26]. These findings suggested that *IL1A* rs1800587 influenced baseline, but not acute, pain in SCD.

Bioinformatics analysis further determined that rs1800587 is located within a DNase cluster containing *cis*-transcriptional regulatory factors [41,42]. Two of 125 ENCODE cell types were affected at this region (chr2:113542806–113543010). H7-human embryonic stem cell (hESC) is a pluripotent cell line with high proliferative potential and greater nucleotide diversity within DNase hypersensitive regions [42]. The other cell line, human tracheal epithelial cell (pHTE), is a primary cell line with limited proliferative potential and lower nucleotide diversity.

Discussion

In this study, we examined the potential influence of *IL1A* rs1800587 SNP on acute and chronic pain in patients with SCD. An innovative pain phenotyping method,

the CPI, taken during routine clinic visits, was used to quantitatively and qualitatively record chronic pain on noncrisis days in patients with SCD. On the other hand, emergency or urgent care utilization due to sickle cell crisis pain was used as a surrogate marker for acute pain. We found that *IL1A* T allele was associated with a significant increase (3.85 points) in CPI score. This study identified an association between chronic pain with a proinflammatory cytokine *IL1A* SNP (rs1800587) in SCD. Our findings also independently confirm previous findings of an association between this SNP and other very different types of chronic pain, namely chronic lumbar radicular pain and low back pain [24,25,43].

IL-1 α has been proposed as a pro-inflammatory cytokine in the IL-1 family [15]. The *IL1A* T allele has been found to possess increased transcriptional activity with enhanced gene expression [23]. Our study also shows that this SNP lies within a DNase cluster, which is commonly found to contain *cis*-regulatory elements such as enhancers, promoters and silencers [42], but these elements have not been demonstrated for this locus so far. It is in agreement with previous studies that found this SNP has regulatory properties on *IL1A* gene expression [44]. Patients with the *IL1A* T allele may have higher levels of IL-1 α in circulation with more intense inflammatory response.

IL-1 α is reported to be more potent than IL-1 β in inducing substance P expression in rat adult sensory neurons [21], which could potentially lower pain threshold and increase pain sensitivity. It is previously observed that immunoreactivity of substance P is increased in the skin of sickle cell mice, which was accompanied by hyperalgesia [45]. Another study shows that IL-1 α level is elevated in the superficial dorsal horn of the spinal cord, a major area for pain transmission and processing, after painful facet joint injury [22]. In addition, in a rat neuropathic pain model there was a significant elevation of IL-1 α mRNA and protein level in dorsal root ganglion but not spinal cord, and intrathecal administration of IL-1 α has anti-allodynic and

Table 4. Negative binomial regression model evaluating the effect of *IL1A* rs1800587 on acute crisis pain and ordinal logistic regression model on utilization groups.

Model	Acute crisis pain		Utilization	
	IRR (95% CI) [†]	p-value	Estimate (95% CI) [†]	p-value
Additive	0.93 (0.70–1.23)	0.606	-0.02 (-0.54–0.51)	0.949
Dominant	0.93 (0.55–1.56)	0.774	0.22 (-0.75–1.20)	0.652
Recessive	0.90 (0.60–1.35)	0.605	-0.18 (-0.94–0.58)	0.637

The major alleles are the reference genotypes in the analyses. Ordered log-odds estimate in which the minor allele would result in a higher utilization group. Regression models are adjusted for age, sex, ethnicity and sickle cell type. Low utilization group (1–3 utilizations), high utilization group (4–38 utilizations).

[†]IRR and 95% CI.

[‡]Ordered log-odds estimate and 95% CI.

IRR: Incident rate ratio.

antihyperalgesic effects [46]. Taken together, IL-1 α is believed to play an important role in the development of neuropathic pain symptoms, although the exact mechanisms are still unclear.

Several clinical reports associated the *IL1A* genotype with chronic pain in patients. The *IL1A* CT/TT carriers in a Norwegian study were reported to have increased risk of chronic lumbar radicular pain after disc herniation [24,43]. The *IL-1 α* gene T allele was associated with increased pain intensity and corresponding reduced pressure pain thresholds in patients with symptomatic disk herniation [24]. Additionally, a possible association of *IL1A* T allele and low back pain has been suggested by a Finnish cohort [25]. Carriers of the *IL1A* T and in combination with the *IL-IRN A*⁻¹⁸¹² allele have an increased risk of, more days and higher pain intensity of low back pain than noncarriers. In our SCD cohort, we found that the T allele was associated with increased CPI, a measurement for the baseline chronic pain, which is consistent with the previous findings. Utilization was used in this study to characterize acute pain phenotypes in SCD patients. However, the *IL1A* T allele does not alter odds of utilization, suggesting that carrying *IL1A* T allele does not increase the rate of acute pain crisis. The outcomes from this study may be limited by the sample. Results will need to be replicated in future studies.

Conclusion

Our study demonstrates that *IL1A* polymorphism rs1800587 is associated with chronic pain in patients with SCD. *IL1A* T allele increases CPI score by 3.85, indicative of increased baseline chronic pain, while *IL1A* T allele does not affect the rate of acute pain crisis. This finding will provide insights into the heterogeneity and prospective genetics studies for pain in SCD.

Future perspective

Pain is prevalent and debilitating in SCD patients with high variability and heterogeneity. The underlying contributors and mechanisms of pain in SCD are poorly understood. Our study implicated the possible involvement of inflammatory factors in the pathogenesis of pain in SCD. Future pain genetics and pharmacological studies can be designed to further unravel the role of inflammation in sickle cell pain. Studying the contributions of *IL1A* and other relevant gene polymorphisms may shed light on the etiology of the pain heterogeneity, allowing for precision pain medicine in SCD.

Financial & competing interests disclosure

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

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.

Executive summary

- A cohort of patients with sickle cell disease (SCD; 97% African-American, mean age of 34.1 years) was studied for the association of *IL1A* polymorphism rs1800587 (located within a DNase cluster containing cis-transcriptional regulatory factors) and pain.
- Composite pain index (CPI) collected during routine clinic visits was used to record chronic pain on non-crisis days, and acute care or emergency department utilization due to sickle cell crisis pain was used as a surrogate marker for acute pain.
- The T allele of *IL1A* rs1800587 was associated with an increase of 3.85 in CPI score in an additive multiple linear regression model, accounting for 3% of the CPI variability.
- There was no statistical association between rs1800587 and utilization in additive, dominant, or recessive multiple linear models.
- The results suggested that *IL1A* rs1800587 influenced baseline chronic pain, but not acute pain, in sickle cell disease.
- Studying the contributions of *IL1A* and other relevant gene polymorphisms may shed light on the etiology of the pain heterogeneity, allowing for precision pain medicine in sickle cell disease.

References

- 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).
- 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).
- Ingram VM. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. *Nature* 178(4537), 792–794 (1956).
- Brousseau DC, Owens PL, Mosso AL, Panepinto JA, Steiner CA. Acute care utilization and rehospitalizations for sickle cell disease. *JAMA* 303(13), 1288–1294 (2010).
- Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology, and prognostic significance. *Am. J. Hematol.* 79(1), 17–25 (2005).
- Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 376(9757), 2018–2031 (2010).
- Smith WR, Penberthy LT, Bovbjerg VE *et al*. Daily assessment of pain in adults with sickle cell disease. *Ann. Intern. Med.* 148(2), 94–101 (2008).
- Vichinsky EP, Johnson R, Lubin BH. Multidisciplinary approach to pain management in sickle cell disease. *Am. J. Pediatr. Hematol. Oncol.* 4(3), 328–333 (1982).
- Marsh S, King CR, Van Booven DJ, Revollo JY, Gilman RH, Mcleod HL. Pharmacogenomic assessment of Mexican and Peruvian populations. *Pharmacogenomics* 16(5), 441–448 (2015).
- Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu. Rev. Pharmacol. Toxicol.* 43, 381–411 (2003).
- Hocking LJ, Smith BH, Jones GT, Reid DM, Strachan DP, Macfarlane GJ. Genetic variation in the beta2-adrenergic receptor but not catecholamine-*O*-methyltransferase predisposes to chronic pain: results from the 1958 British Birth Cohort Study. *Pain* 149(1), 143–151 (2010).
- Bandeira IC, Rocha LB, Barbosa MC *et al*. Chronic inflammatory state in sickle cell anemia patients is associated with HBB(*)S haplotype. *Cytokine* 65(2), 217–221 (2014).
- Sarray S, Mahdi N, Saleh LR, Almaoui WY. Reduction in serum IL-10 levels is a surrogate marker for predicting vaso-occlusive crisis in sickle cell disease. *Am. J. Hematol.* 89(7), 789–790 (2014).
- Keikhaei B, Mohseni AR, Norouzirad R *et al*. Altered levels of pro-inflammatory cytokines in sickle cell disease patients during vaso-occlusive crises and the steady state condition. *Eur. Cytokine. Netw.* 24(1), 45–52 (2013).
- Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci. Lett.* 361(1–3), 184–187 (2004).
- Wolf G, Livshits D, Beilin B, Yirmiya R, Shavit Y. Interleukin-1 signaling is required for induction and maintenance of postoperative incisional pain: genetic and pharmacological studies in mice. *Brain Behav. Immun.* 22(7), 1072–1077 (2008).
- Rodrigues DH, Leles BP, Costa VV *et al*. IL-1beta is involved with the generation of pain in experimental autoimmune encephalomyelitis. *Mol. Neurobiol.* 53(9), 6540–6547, 2016.
- Del Rey A, Yau HJ, Randolph A *et al*. Chronic neuropathic pain-like behavior correlates with IL-1beta expression and disrupts cytokine interactions in the hippocampus. *Pain* 152(12), 2827–2835 (2011).
- Lipworth BJ, Basu K, Donald HP *et al*. Tailored second-line therapy in asthmatic children with the Arg(16) genotype. *Clin. Sci. (London)* 124(8), 521–528 (2013).
- Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of *ADRB2* polymorphisms on response to longacting beta2-agonist therapy: a pharmacogenetic analysis of two randomised studies. *Lancet* 370(9605), 2118–2125 (2007).
- Skoff AM, Zhao C, Adler JE. Interleukin-1alpha regulates substance P expression and release in adult sensory neurons. *Exp. Neurol.* 217(2), 395–400 (2009).
- Kras JV, Dong L, Winkelstein BA. Increased interleukin-1alpha and prostaglandin E2 expression in the spinal cord at 1 day after painful facet joint injury: evidence of early spinal inflammation. *Spine (Phila Pa 1976)* 39(3), 207–212 (2014).
- Zuurhout MJ, Vijverberg SJ, Raaijmakers JA *et al*. Arg16 *ADRB2* genotype increases the risk of asthma exacerbation in children with a reported use of long-acting beta2-agonists: results of the PACMAN cohort. *Pharmacogenomics* 14(16), 1965–1971 (2013).
- Schistad EI, Jacobsen LM, Roe C, Gjerstad J. The interleukin-1alpha gene C>T polymorphism rs1800587 is associated with increased pain intensity and decreased pressure pain thresholds in patients with lumbar radicular pain. *Clin. J. Pain* 30(10), 869–874 (2014).
- Solovieva S, Leino-Arjas P, Saarela J, Luoma K, Raininko R, Riihimaki H. Possible association of interleukin 1 gene locus polymorphisms with low back pain. *Pain* 109(1–2), 8–19 (2004).
- Ezenwa MO, Molokie RE, Wang ZJ *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).
- Wilkie DJ, Molokie RE, Suarez ML, Ezenwa MO, Wang ZJ. Composite pain index: reliability, validity, and sensitivity of a patient-reported outcome for research. *Pain Med.* 16(7), 1341–1348 (2015).
- Melzack R. The McGill pain questionnaire: major properties and scoring methods. *Pain* 1, 277–299 (1975).
- Ngamkham S, Holden JE, Wilkie DJ. Differences in pain location, intensity, and quality by pain pattern in outpatients with cancer. *Cancer Nurs.* 34(3), 228–237 (2011).
- Wilkie DJ, Judge MK, Berry DL, Dell J, Zong S, Gillespie R. Usability of a computerized PAINReportIt in the general public with pain and people with cancer pain. *J. Pain Symptom Manage.* 25(3), 213–224 (2003).
- Jhun E, He Y, Yao Y, Molokie RE, Wilkie DJ, Wang ZJ. Dopamine D3 receptor Ser9Gly and catechol-*O*-

- methyltransferase Val158Met polymorphisms and acute pain in sickle cell disease. *Anesth. Analg.* 119(5), 1201–1207 (2014).
- 32 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16(3), 1215 (1988).
- 33 Vandenbergh DJ, Anthony K, Whitfield KE. Optimizing DNA yield from buccal swabs in the elderly: attempts to promote buccal cell growth in culture. *Am J. Hum. Biol.* 15(5), 637–642 (2003).
- 34 Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet.* Chapter 2, Unit 2, 12 (2009).
- 35 Lettre G, Lange C, Hirschhorn JN. Genetic model testing and statistical power in population-based association studies of quantitative traits. *Genet. Epidemiol.* 31(4), 358–362 (2007).
- 36 Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case–control studies. *Nat. Protoc.* 6(2), 121–133 (2011).
- 37 Cameron A, Trivedi PK. New York, NY. *Regression Analysis of Count Data (1st Edition)*. Cambridge University Press (1998).
- 38 Negative Binomial Regression. *SPSS Data Analysis Examples* (23 November 2015). www.ats.ucla.edu/stat/spss/dae/neg_binom.htm
- 39 Kent WJ, Sugnet CW, Furey TS *et al.* The human genome browser at UCSC. *Genome Res.* 12(6), 996–1006 (2002).
- 40 Encyclopedia of DNA Elements at UCSC. <https://genome.ucsc.edu/ENCODE/>
- 41 Rosenbloom KR, Sloan CA, Malladi VS *et al.* ENCODE data in the UCSC genome browser: year 5 update. *Nucleic Acids Res.* 41(Database issue), D56–D63 (2013).
- 42 Thurman RE, Rynes E, Humbert R *et al.* The accessible chromatin landscape of the human genome. *Nature* 489(7414), 75–82 (2012).
- 43 Moen A, Schistad EI, Rygh LJ, Roe C, Gjerstad J. Role of *IL1A* rs1800587, *IL1B* rs1143627 and *IL1RN* rs2234677 genotype regarding development of chronic lumbar radicular pain; a prospective one-year study. *PLoS ONE* 9(9), e107301 (2014).
- 44 Dominici R, Cattaneo M, Malferrari G *et al.* Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 alpha. *Immunogenetics* 54(2), 82–86 (2002).
- 45 Kohli DR, Li Y, Khasabov SG *et al.* Pain-related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: modulation by cannabinoids. *Blood* 116(3), 456–465 (2010).
- 46 Mika J, Korostynski M, Kaminska D *et al.* Interleukin-1 alpha has antiallodynic and antihyperalgesic activities in a rat neuropathic pain model. *Pain* 138(3), 587–597 (2008).