Published in final edited form as:

Blood Cells Mol Dis. 2015 February ; 54(2): 155–159. doi:10.1016/j.bcmd.2014.11.016.

Modulation of pain in pediatric sickle cell disease: understanding the balance between endothelin mediated vasoconstriction and apelin mediated vasodilation

Terika P. Smith¹, Alyssa M. Schlenz², Jeffrey C. Schatz², Rangan Maitra³, and Sarah M. Sweitzer^{1,4}

¹Department of Pharmacology, Physiology and Neuroscience, University of South Carolina, Columbia, SC.

²Department of Psychology, University of South Carolina, Columbia, SC.

³RTI International, Research Triangle Park, NC

⁴Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, Clinton, SC.

Abstract

Children with sickle cell disease (SCD) have painful vaso-occlusive episodes (VOE), which often reoccur across the individual's lifespan. Vaso-constrictive and vaso-dilatory molecules have been hypothesized to play a role in VOEs. Endothelin-1 (ET-1) is a potent vasoconstrictor that is released during VOEs and is correlated with pain history. Apelin is a vaso-dilatory peptide that also has a modulatory role in pain processing. We hypothesize that the ratio between vaso-dilatory and vaso-constrictive tone in children with SCD may be a marker of pain sensitization and vaso-occlusion. Plasma endothelin and apelin levels were measured in 47 children with SCD. Procedural and baseline pain were assessed via child- and caregiver-reports and observational distress. Pain history was assessed using retrospective chart review. Plasma apelin was related to age, with decreased levels in older children. The ratio between apelin and ET-1 was negatively correlated to observational baseline pain. The ratio between apelin and Big ET was negatively correlated to caregiver ratings of baseline pain and positively correlated to history of VOEs, which is possibly due to hydroxyurea treatment. These results suggest that an imbalance in the apelin and endothelin systems may contribute to an increasing number of VOEs and baseline pain in children with SCD.

Keywords

sickle cell disease; apelin; endothelin; vaso-occlusive episodes; pain

© 2014 Elsevier Inc. All rights reserved.

Corresponding author information: 6439 Garners Ferry Road, Columbia, SC 29209, USA Phone: +1 803 216 3526 Fax: +1 803 216 3538 terika.smith@uscmed.sc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Sickle cell disease (SCD) is a genetic blood disorder that is characterized by recurrent vasoocclusive events (VOE) during which sickle-shaped red blood cells occlude small blood vessels. One of the major characteristics of VOE is severe pain that often peaks at the height of the VOE and then slowly returns to normal over the course of days to weeks. It has been suggested that in SCD, there may be an imbalance in the production of local vasoconstrictors and vasodilators, with a shift in the balance towards the production of vasoconstrictors [1]. One of these vasoconstrictors thought to be involved is endothelin-1 (ET-1), one of the most potent endogenous vasoconstrictors. Studies have shown that ET-1 levels are increased in patients with SCD compared to healthy controls [2, 3] and these increased levels are even more pronounced during an acute VOE [1]. Down-regulation of ET-1 gene expression and a reduction in the half-life of circulating ET-1 has been found in patients with SCD treated with hydroxyurea (HU), which is a drug that has been shown to decrease the rate and intensity of VOEs [4-6]. In addition to its vasoactive properties, ET-1 also causes nociception when injected into both humans and animals [7-13]. Interestingly, the time course of ET-1 plasma elevations parallel VOE-associated pain symptoms with a peak in plasma levels and pain at the height of the VOE and then slow return to baseline that requires several weeks [3].

Previously, we have shown that endothelin variables in the plasma of pediatric patients with SCD is related to baseline pain such that higher plasma levels of ET-1 and its precursor, Big ET, were found in children with higher baseline pain [14]. In contrast, lower plasma Big ET levels were found in children with a higher frequency of recent VOEs, possibly a result of recent conversion to vasoactive ET-1. From these findings of excessive ET mediated vasoconstriction in children with SCD we were interested in the role of vasodilatory systems in SCD. We have recently begun to explore the role of apelin, a novel vasoactive peptide with vasodilatory properties, in the pain associated with SCD. Apelin is formed from the 77 amino acid preproapelin, which is cleaved by angiotensin-converting enzyme 2 to form apelin-36 and other biologically active fragments such as apelin-17 and apelin-13 [15, 16]. Apelin is the endogenous ligand for the orphan G-protein coupled receptor, APJ. The apelin-APJ system has also been recently implicated in having a modulatory role in nociception since apelin and its receptor are found in several brain regions associated with pain [17]. In the vasculature, apelin's interaction with its receptor causes nitric-oxide (NO) dependent vasodilation when acting on endothelial cells [16, 18, 19]. Recently, it has been proposed that there is a shift in favor of ET-1-mediated vasoconstriction and away from NO-mediated vasodilation during an acute VOE in SCD patients [1]. To date, there have been no studies that explore the potential relationship between apelin and ET-1 in relation to pain in SCD.

The goal of this study was to examine the balance between ET-1 or its precursor, Big ET, and apelin in a cohort of pediatric patients with SCD and how this balance correlates with acute chest syndrome, procedural pain, and pain history. No directional hypotheses are offered as this is the first known study to evaluate apelin in relation to SCD outcomes and both positive and negative associations could be justified based on existing data.

2. Methods

2.1 Participants - Apelin plasma

This study used a subsample of 47 children with SCD ages 2 to 18 ($M = 9.98$, $SD = 4.78$; 22 male, 25 female) that were participating in a larger study of procedural pain in SCD. Due to the goals of the larger study, children between the ages of 10 and 12 (the transition from pre- to post-pubescence) were excluded. All protocols were approved by the Institutional Review Board of Palmetto Richland Hospital, which provides approval concomitantly with the University of South Carolina. All participants were recruited from a Hematology Clinic located in Columbia, South Carolina over a 9-month period. Forty-seven children had adequate plasma available for apelin analysis by ELISA. For the ratios, 46 children had both ET-1 and apelin data and 43 children had both Big ET and apelin data available. The ET-1 and Big ET ELISAs were conducted as part of the previous study and details about those results can be found in Schlenz, et al. [14]. For child ratings discussed below, only children over the age of 5 completed ratings, resulting in a sample of 37 children for the apelin/ET-1 analysis and 36 children for the apelin/Big ET analysis.

2.2 Procedures

Children and their caregivers were approached at routine hematologist visits for participation. Children routinely receive venipuncture at these visits. Venipuncture was chosen to represent a standardized painful stimulus. After consent and assent procedures were completed, children and caregivers completed baseline (pre-venipuncture) ratings of pain and caregivers completed a background questionnaire. Once the venipuncture was completed, children and caregivers completed ratings of the child's pain during the procedure. Children were video recorded from the time they entered the exam room to the end of the venipuncture, in order to obtain observational ratings of pain. Medical record reviews were conducted after the child's visit using a structured coding method.

2.3 Measures

Caregivers completed a background information questionnaire to obtain demographic information. Children rated their pain using the Wong Baker Faces Scale [20]. Caregivers rated their child's pain using a visual analog scale (VAS). Observational ratings of pain were also taken at baseline and during the procedure using the modified version of the Observational Scale of Behavioral Distress [21]. Reviews of children's medical charts were used to establish history of acute chest syndrome, hydroxyurea status, and VOs. Of the 47 children in this sample, 12 had a history of acute chest syndrome and 35 did not. For recent VOE history, we measured the number of hospitalizations, emergency department visits, and outpatient contacts for pain in the previous 24 months.

2.4 ELISA

Blood collection and plasma separation for this sample of participants has been described previously [14]. Briefly, blood was collected into EDTA vacutainer tubes and placed on ice for plasma isolation within 30 minutes of blood collection. After isolation, plasma was stored at -80°C until further analysis by ELISA. ELISA kits for ET (1-21) (Cat no.

BI-20052) and Big ET (Cat no. BI-20082) were purchased from ALPCO Immunoassays and apelin-36 (EK-057-15), which recognizes apelin-12, -13, and -36, was purchased from Phoenix Pharmaceuticals. ELISAs for ET-1 and Big ET-1 were performed in triplicate and ELISAs for apelin-36 were performed in duplicate according to the respective assay protocols. A standard curve was plotted from the standards of each kit using Prism software (GraphPad Software Inc, San Diego, CA), which was then used to extrapolate the sample concentrations from each plasma sample.

2.5 Data Analysis

This study used an exploratory analysis to examine relationships between apelin and ratios of apelin to ET-1 and Big ET to the primary outcomes: baseline pain, procedural pain, acute chest syndrome, recent VOE history, and hydroxyurea status. We were particularly interested in providing effect sizes that could be used for future research in this area. For VOE history, there was one outlier (a child with 58 documented pain episodes) who was removed from analysis. The relationships between apelin, the two ratios, baseline pain, procedural pain, and VOE history were assessed using correlations. The relationship between apelin, the two ratios, acute chest syndrome, and hydroxyurea status were assessed via t-test. Descriptive information on age and gender differences for apelin is also provided using correlation and t-test analysis, respectively. Information on age and gender for ET-1 and Big ET can be found in a prior publication [14].

The ratio variables demonstrated a positive skew that was corrected with log-transformation. Additionally, to ensure that the ratios were equally associated with both apelin and the endothelin variables, we examined correlations between the ratios, apelin, ET-1, and Big ET. The log-transformed ratio for apelin/ET-1 was highly associated with ET-1 ($r = -.84$) whereas the untransformed ratio was more equally associated with both apelin ($r = .41$) and ET-1 ($r = -.47$). We reported the untransformed results for this ratio descriptively below; however, both the log-transformed and untransformed results can be found in Table 1. For outcome variables, the three baseline and procedural pain ratings all demonstrated a positively skewed distribution and were log-transformed. In addition, because baseline ratings of pain were associated with significantly greater procedural pain, regression was used to remove variance in procedural pain that could be explained by baseline ratings. This approach allowed for the procedural pain ratings to solely reflect pain from the procedure. Finally, due to the exploratory nature of this study, we were careful to evaluate the impact of outliers (defined as values exceeding three standard deviations from the mean of the variable).

3. Results

Table 1 provides all correlation results and these findings are explained descriptively below. T-test results are reported here.

3.1 Apelin

Apelin levels ranged from 0.87-6.94 ng/mL and were significantly related to age, with older age associated with lower apelin levels. There was no statistically significant difference in

mean apelin levels between male ($M = 2.53$, $SD = 1.42$) and female ($M = 2.61$, $SD = 1.12$) children, $t(45) = -.22$, $p = .829$.

There was no statistically significant difference in apelin levels between children with ($M = 2.20$, $SD = .91$) and without ($M = 2.71$, $SD = 1.37$) a history of acute chest syndrome, $t(45) = -1.24$, $p = .221$. Apelin levels alone were not significantly related to baseline pain, procedural pain, or recent VOE history.

3.2 Apelin/ET-1 Ratio (log-transformed)

There was no statistically significant difference in apelin/ET-1 ratio values for children with ($M = 5.87$, $SD = 5.58$) and without ($M = 4.22$, $SD = 4.11$) a history of acute chest syndrome $t(44) = 1.09$, $p = .282$. The apelin/ET-1 ratio was not significantly related to procedural pain or recent VOE history. However, the apelin/ET-1 ratio was negatively correlated to observational baseline pain ratings ($r = -.32$, $p = .032$).

3.3 Apelin/Big ET Ratio (log-transformed)

There was no significant difference in apelin/Big ET ratio values for children with ($M = .93$, $SD = .35$) and without ($M = .79$, $SD = .36$) a history of acute chest syndrome $t(41) = 1.15$, $p = .257$. The apelin/Big ET ratio was significantly associated with caregiver ratings of baseline pain ($r = -.30$, $p = .049$) and approached significance in relation to child ratings of baseline pain ($r = -.29$, $p = .088$). The apelin/Big ET ratio was not significantly related to the remaining baseline and procedural pain variables or VOE history.

Outliers. The same outlier noted above for VOE history was removed, resulting in a statistically significant correlation for the apelin/Big ET ratio and VOE history, $r = .32$, $p = .041$ (Figure 1). There was also a trend for a significantly higher ratio of apelin to Big ET in children who were currently on hydroxyurea treatment compared to children who were not on this treatment, $p = 0.082$ (Figure 2).

4. Discussion

Previous work has shown that endothelin variables were more closely associated with baseline pain in children with SCD [14], and in this study, we sought to examine apelin levels in relation to endothelin variables in the same sample of children. We found that plasma apelin expression was related to age, with decreased levels as a child ages, but no relationship was found between apelin and baseline pain, procedural pain, or history of recent VOEs. Similarly, the ratio between apelin and ET-1 was not related to procedural pain or recent VOEs, but it was negatively correlated to observational baseline pain. The ratio between apelin and Big ET was negatively correlated with caregiver ratings of baseline pain. In contrast, the ratio between apelin and Big ET was found to positively correlate to history of VOEs such that increased ratios of the two peptides was associated with an increased number of VOEs. We also found that there was a trend for a relationship between hydroxyurea status and the ratio between apelin and Big ET. The results from this study suggest a relationship between the ratio between the apelin and endothelin systems and the number of VOEs and baseline pain before venipuncture. This is the first study that has

demonstrated a potential relationship between the apelin and endothelin systems in one of the hallmark complications associated with SCD.

4.1 Impairment in regulation of vascular tone in SCD

Vascular tone is maintained by a delicate balance of vasoconstrictors and vasodilators, and in diseases like SCD, it is believed that there is an imbalance in these systems that may further contribute to vaso-occlusion and the pain associated with vaso-occlusion [1, 22]. Our previous work has shown that increased plasma endothelin levels were more closely associated with greater baseline pain in children with SCD [14]. In humans, intravenous infusion of apelin into arteries causes endothelial NO-mediated vasodilation [18]. In the vasculature, binding of apelin to the APJ receptor produces NO dependent vasodilation [18, 19]. Impairment in endothelium-dependent vasodilation with decreased NO production under basal conditions and during times of wall shear stress such as experienced during a VOE has been reported in patients with SCD [23]. These current findings are the first to suggest that impairment in endothelium-dependent vasodilation in SCD may be the result of an imbalance between ET-1-mediated vasoconstriction and apelin-mediated vasodilation.

In the current study, the ratio of apelin to Big ET was positively correlated with a higher frequency of VOEs. An increase in the ratio can result from increased apelin levels or decreased Big ET levels. We have previously shown that lower plasma levels of Big ET were correlated with more recent VOEs [14]. These results are the opposite of what we would have expected. Since we found a trend for a relationship between hydroxyurea status and the apelin/Big ET ratio, we predict that hydroxyurea treatment is lowering Big ET plasma levels, thereby increasing the ratio value in children with higher frequencies of VOEs. Another explanation is that the lower levels of Big ET could result from an increased conversion of Big ET into the more active peptide, ET-1, by endothelin converting enzyme-1.

4.2 Apelin in SCD

In human endothelial and vascular smooth muscle cells exposed to hypoxic conditions, apelin expression is significantly up-regulated, which may be a compensatory response to low oxygen levels in the tissue [24]. Although the current study did not show a relationship between apelin levels alone and pain history or ratings, there are several limitations to the study design which might contribute to these findings. The first being that apelin was sampled at a single time point from patients during disease steady state. Children who had a recent VOE or were taking current pain medications had been excluded from participating in the study. It is possible that changes in apelin expression may be more pronounced during periods of vascular crisis when vasodilation has been shown to be significantly impaired in SCD patients [23]. Similarly, the imbalance between the apelin and endothelin systems found in this study may be more pronounced during a crisis. In addition, contributions of the apelin system in the current study may be underestimated based on our single sampling method since apelin has a short plasma half-life of less than 8 minutes [18].

4.3 Vaso-regulatory mediators and age-dependence

This study also shows that apelin decreases in age in children with SCD. This decrease in plasma apelin levels with age somewhat parallels the decrease in circulating ET-1 with age in children with SCD [6], which may suggest a developmental role for these mediators in the vaso-regulatory systems in SCD. These results are also consistent with animal studies showing a decrease in plasma apelin levels in aged rats (22 months) compared to young rats (3 months), which may suggest a developmental role for these mediators in the vascular system [25]. It should also be noted that the range of plasma level of apelin found in our study (0.87-6.94 ng/mL) is much higher than levels (0.0493 to 0.273 ng/mL) found in healthy adult volunteers in another study, which may be due to the decrease in apelin levels with age [26]. In addition, ET-1-induced spontaneous nociceptive behaviors in rodents are age dependent [12]. Significantly greater ET-1-induced nociceptive behaviors have been reported in younger animals compared to older animals. Similarly, a priming effect with ET-1 has been characterized in young rodents in which increased ET-1 induced nociceptive behaviors are produced by a second exposure to ET-1 [27]. This is important in certain disease states, such as sickle cell disease, in which there are repeated exposures to ET-1 with each subsequent VOE.

4.4 Limitations

Extrapolation of the current findings to larger patient populations is limited by the small sample size. A larger sample size may reveal significant differences in plasma apelin levels and the ratios between the more severe genotypes and the more mild-moderate genotypes in regards to painful VOEs, since the different genotypes have been shown to influence the frequency of VOEs and pain factors [28]. Another limitation of this study is the short half-lives of plasma apelin and ET-1 and Big ET, which are less than 8 minutes, 1-2 minutes, and 23 minutes, respectively [18, 29, 30].

4.5 Conclusion

This is the first study to explore the potential relationship between apelin and ET-1 in the pain associated with SCD. The current results support the general postulate that children with SCD with higher numbers of VOEs may have a greater imbalance between the vasoconstrictive and pronociceptive systems and vasodilatory and anti-nociceptive systems. Further studies in this area are needed to define and characterize the relationships between the apelin and endothelin systems in pain-associated with SCD. If larger studies confirm that the balance between apelin and endothelin are important in SCD then modulating this target may be a unique therapeutic approach to treat and prevent VOEs.

Acknowledgements

This work was funded by National Institutes of Health grants R01 DA023593 and NS26363 (SMS), and F31 HL108582 (AMS), a South Carolina Post-Baccalaureate Research Education Programs for Minorities funded by grants R25 GM066526 and R25 GM076277 from the National Institutes of Health, and the Alfred P Sloan Foundation (TPS).

References

1. Ergul S, et al. Vasoactive factors in sickle cell disease: in vitro evidence for endothelin-1-mediated vasoconstriction. *Am J Hematol.* 2004; 76(3):245–51. [PubMed: 15224360]
2. Werdehoff SG, et al. Elevated plasma endothelin-1 levels in sickle cell anemia: relationships to oxygen saturation and left ventricular hypertrophy. *Am J Hematol.* 1998; 58(3):195–9. [PubMed: 9662270]
3. Graido-Gonzalez E, et al. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. *Blood.* 1998; 92(7):2551–5. [PubMed: 9746797]
4. Brun M, et al. Hydroxyurea downregulates endothelin-1 gene expression and upregulates ICAM-1 gene expression in cultured human endothelial cells. *Pharmacogenomics J.* 2003; 3(4):215–26. [PubMed: 12931135]
5. Odievre MH, et al. Sodium phenyl butyrate downregulates endothelin-1 expression in cultured human endothelial cells: relevance to sickle-cell disease. *Am J Hematol.* 2007; 82(5):357–62. [PubMed: 17373676]
6. Lapoumeroulie C, et al. Decreased plasma endothelin-1 levels in children with sickle cell disease treated with hydroxyurea. *Haematologica.* 2005; 90(3):401–3. [PubMed: 15749673]
7. Dahlof B, et al. Regional haemodynamic effects of endothelin-1 in rat and man: unexpected adverse reaction. *J Hypertens.* 1990; 8(9):811–7. [PubMed: 2172370]
8. Ferreira SH, Romitelli M, de Nucci G. Endothelin-1 participation in overt and inflammatory pain. *J Cardiovasc Pharmacol.* 1989; 13(Suppl 5):S220–2. [PubMed: 2473319]
9. Hans G, et al. Neurosensory changes in a human model of endothelin-1 induced pain: a behavioral study. *Neurosci Lett.* 2007; 418(2):117–21. [PubMed: 17403578]
10. Gokin AP, et al. Local injection of endothelin-1 produces pain-like behavior and excitation of nociceptors in rats. *J Neurosci.* 2001; 21(14):5358–66. [PubMed: 11438612]
11. Davar G, et al. Behavioral signs of acute pain produced by application of endothelin-1 to rat sciatic nerve. *Neuroreport.* 1998; 9(10):2279–83. [PubMed: 9694215]
12. McKelvy AD, Mark TR, Sweitzer SM. Age- and sex-specific nociceptive response to endothelin-1. *J Pain.* 2007; 8(8):657–66. [PubMed: 17553749]
13. Smith T, et al. Endothelin-1-induced priming to capsaicin in young animals. *Neurosci Lett.* 2014
14. Schlenz AM, et al. Sensitization to acute procedural pain in pediatric sickle cell disease: modulation by painful vaso-occlusive episodes, age, and endothelin-1. *J Pain.* 2012; 13(7):656–65. [PubMed: 22633685]
15. Cheng B, et al. Neuroprotection of apelin and its signaling pathway. *Peptides.* 2012; 37(1):171–3. [PubMed: 22820556]
16. Tatemoto K, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun.* 1998; 251(2):471–6. [PubMed: 9792798]
17. Lv SY, et al. Supraspinal antinociceptive effect of apelin-13 in a mouse visceral pain model. *Peptides.* 2012; 37(1):165–70. [PubMed: 22732665]
18. Japp AG, et al. Vascular effects of apelin in vivo in man. *J Am Coll Cardiol.* 2008; 52(11):908–13. [PubMed: 18772060]
19. Maguire JJ, et al. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension.* 2009; 54(3):598–604. [PubMed: 19597036]
20. Wong DL, Baker CM. Pain in children: comparison of assessment scales. *Pediatr Nurs.* 1988; 14(1):9–17. [PubMed: 3344163]
21. Elliott CH, Jay SM, Woody P. An observation scale for measuring children's distress during medical procedures. *J Pediatr Psychol.* 1987; 12(4):543–51. [PubMed: 3430286]
22. Nath KA, et al. Mechanisms of vascular instability in a transgenic mouse model of sickle cell disease. *Am J Physiol Regul Integr Comp Physiol.* 2000; 279(6):R1949–55. [PubMed: 11080057]
23. Belhassen L, et al. Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation. *Blood.* 2001; 97(6):1584–9. [PubMed: 11238095]

24. Eyries M, et al. Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. *Circ Res*. 2008; 103(4):432–40. [PubMed: 18617693]
25. Sauvant J, et al. Mechanisms involved in dual vasopressin/apelin neuron dysfunction during aging. *PLoS One*. 2014; 9(2):e87421. [PubMed: 24505289]
26. Zhen EY, Higgs RE, Gutierrez JA. Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. *Anal Biochem*. 2013; 442(1):1–9. [PubMed: 23872001]
27. McKelvy AD, Sweitzer SM. Endothelin-1 exposure on postnatal day 7 alters expression of the endothelin B receptor and behavioral sensitivity to endothelin-1 on postnatal day 11. *Neurosci Lett*. 2009; 451(1):89–93. [PubMed: 19111903]
28. Platt OS, et al. Pain in sickle cell disease. Rates and risk factors. *N Engl J Med*. 1991; 325(1):11–6. [PubMed: 1710777]
29. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res*. 2004; 61(2):227–37. [PubMed: 14736539]
30. Hemsén A, et al. Metabolism of Big endothelin-1 (1-38) and (22-38) in the human circulation in relation to production of endothelin-1 (1-21). *Regul Pept*. 1995; 55(3):287–97. [PubMed: 7761628]

Figure 1. Positive correlation for the ratio between apelin and Big ET and recent VOEs
Higher ratio of apelin to Big ET is significantly associated with a higher number of recent VOEs after removal of outlier ($N = 42$).

Figure 2. Relationship between hydroxyurea status and the ratio between apelin and Big ET
There was a trend for a significant difference in the apelin/Big ET ratio between children who are currently on hydroxyurea and children who are not. Children who are currently on hydroxyurea had a higher apelin/Big ET ratio compared to children who were not currently taking hydroxyurea ($p=0.082$).

Table 1

Correlation Results for Demographics, Apelin, Ratios, baseline and procedural pain, and VOE History

Variables	1	2	3	4	5	6	7	8	9	10	11
1. Age	-	-.29*	-.02	.07	-.29 [†]	-.07	-.42**	-.35	-.30*	-.49**	.36*
2. Apelin	-.29*	-	.29*	.51**	-.14	-.03	.03	.03	-.01	.21	-.19
3. Apelin/ET-1 Ratio	.05	.41**	-	.61**	-.24	-.12	-.32*	.05	-.11	.03	.16
4. Apelin/Big ET Ratio	.25 [†]	.43**	.74**	-	-.29 [†]	-.30*	-.23	-.07	-.22	.04	.23
5. Child Report (Baseline Pain)	-.29 [†]	-.14	-.25	-.22	-	.53**	.21	-.08	.09	-.06	-.14
6. Caregiver Report (Baseline Pain)	-.07	-.03	-.20	-.20	.53**	-	-.01	.28	.00	.19	-.01
7. Obs. Distress (Baseline Distress)	-.42**	.03	-.24	-.20	.21	-.01	-	.24	.24	.08	-.19
8. Child Report (Procedural Pain)	-.35*	.03	-.10	-.23	-.08	.28	.24	-	.27	.37*	-.13
9. Caregiver Report (Procedural Pain)	-.30*	-.01	-.14	-.35*	.09	.00	.24	.27	-	.18	-.21
10. Obs. Distress (Procedural Distress)	-.49***	.21	.03	-.10	-.06	.19	.08	.37*	.18	-	.00
11. Recent VOEs	.36*	-.19	.18	.27 [†]	-.14	-.01	-.19	-.13	-.21	.00	-

Note. The upper diagonal displays correlations with log-transformed ratio values whereas the lower diagonal displays correlations with untransformed ratio values.

[†] p<0.10

* p<0.05

** p<0.01

*** p<0.001