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TITLE: VIPER: Chronic Pain after Amputation: Inflammatory Mechanisms, Novel Analgesic Pathways, and Improved Patient Safety

PRINCIPAL INVESTIGATOR: Thomas Van de Ven MD, PhD

CONTRACTING ORGANIZATION: Duke University

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Thomas Van de Ven MD, PhD

E-Mail: thomas.vandeven@duke.edu

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Duke University
2200 W Main St Ste 710
Durham, NC 27705

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14. ABSTRACT
Chronic pain is a significant problem after nerve injury from trauma or surgery. Current therapies and attempts at prevention have proven largely ineffective. Through analysis of data obtained in the Molecular Signatures of Chronic Pain Subtypes study termed Veterans Integrated Pain Evaluation Research (VIPER) (W81XWH-11-2-0003) we have discovered two novel pain pathways with potential therapeutic relevance (Wnt and TGR5). In addition, we recognize that improving the safety and efficacy of existing therapies must continue to be a priority and plan to use the large pharmacogenomic database at Vanderbilt University to identify patients at risk for adverse opioid related events. The current proposal intends to study the contribution of non-neuronal immune cells (macrophages) to chronic pain while also evaluating novel analgesics in relevant animal models. The current proposal also attempts to determine the optimal patient population for opioid therapy while identifying those patients at greatest risk from opioids.

15. SUBJECT TERMS
Post-amputation pain, Phantom limb pain, Residual limb pain, neuropathic pain, novel analgesics, opioid related adverse events.

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INTRODUCTION:
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KEYWORDS:
Post-amputation pain, Phantom limb pain, Residual limb pain, neuropathic pain, novel analgesics, opioid related adverse events.

Major goals of this research project

Specific Aim 1: Characterize the role of *Wnt* signaling in macrophage polarization, mouse nerve injury models and human neuroinflammation

Major Task 1: Characterize macrophage polarization changes after Wnt signaling modification in mouse macrophage cell culture.

Major Task 2: Determine the specific wnt pathway responsible for prevention of mechanical allodynia in a mouse model of peripheral nerve injury and correlate this with macrophage polarization state and IL-6 to IL-10 ratio.

Major Task 3: Characterize wnt pathway expression and DNA methylation changes in humans before and after amputation and determine the role of cytokine ratio measurement in prediction of pain phenotype.

Specific Aim 2: Determine the role of TGR5 in astrocyte activation and treatment of mechanical allodynia in a mouse model of neuropathic pain.

Major Task 1: Determine role of TGR5 signaling in astrocyte activation

Major Task 2: Determine the role of TGR5 signaling in treating mechanical allodynia in a mouse peripheral nerve injury model

Specific Aim 3: Use existing data from the Vanderbilt EMR and genotyping repositories to look for associations between genetic variants and pain phenotypes

Major Task 1: Preliminary analyses conducted to confirm the precise numbers of patients for whom there are sufficient data available. Validation of previously published genotype-phenotype associations.

Major Task 2: Discovery and validation of novel exomic variants associated with opioid adverse drug events.

What was accomplished under these goals?

Overall Results

Specific Aim 1: Characterize the role of *Wnt* signaling in macrophage polarization, mouse nerve injury models and human neuroinflammation

Major Task 1: Characterize macrophage polarization changes after Wnt signaling modification in mouse macrophage cell culture.

Summary of Results of Major Task 1: Non-canonical wnt activation leads to M2 phenotype in cultured macrophages. The M2 phenotype is associated with reduced inflammation. Therefore, non-canonical wnt pathway activators may prevent the neuroinflammation that causes chronic neuropathic pain.

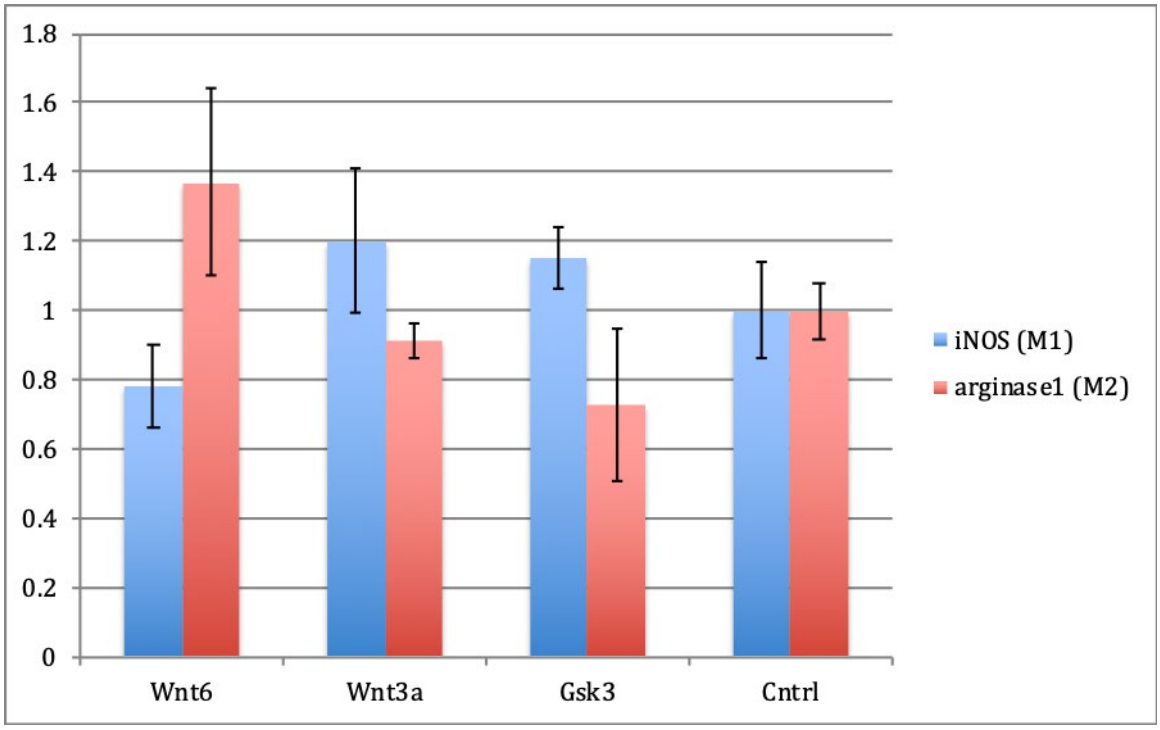

Figure 1) Non-canonical wnt ligand Wnt6 favors M2 macrophage phenotype. Murine peritoneal macrophages were collected and cultured and treated with either wnt pathway ligands (wnt6 100ng/ml, wnt3a 100ng/ml, gsk3 inhibitor 100ng/ml or saline as control). RNA was collected and qPCR performed. Transcript levels were first normalized to GAPDH as a reference gene, and then to control for comparison to various treatments. All data are mean ± SEM (n=3, treatments and control).

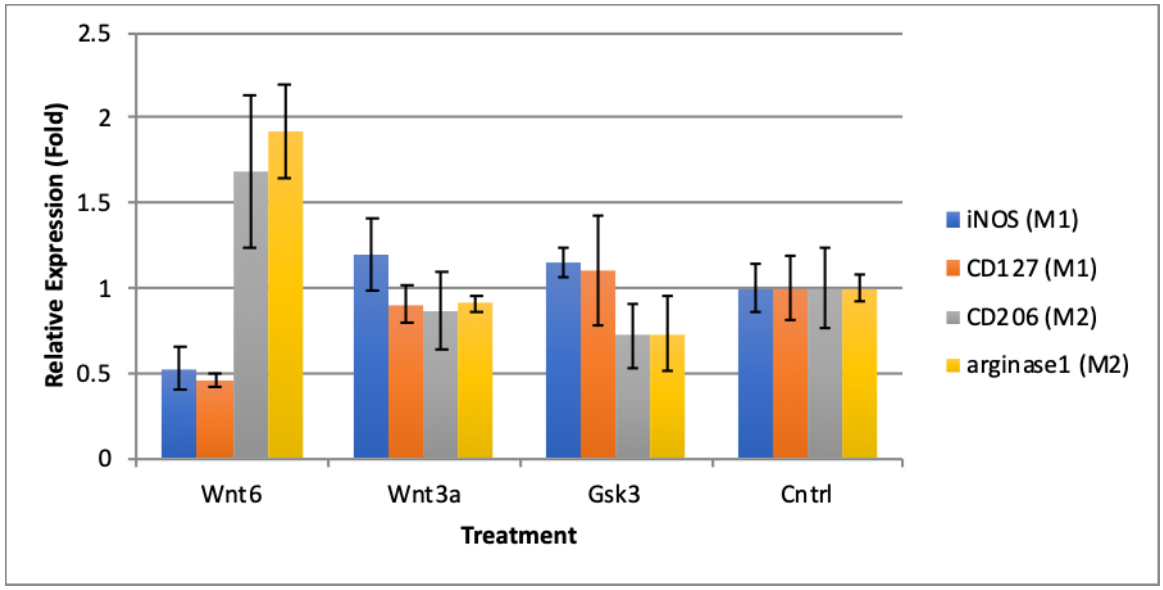

Figure 2) Non-canonical wnt ligand Wnt6 favors M2 macrophage phenotype. Murine peritoneal macrophages were collected and cultured and treated with either wnt pathway ligands (wnt6 100ng/ml, wnt3a 100ng/ml, gsk3 inhibitor 100ng/ml or saline as control). RNA was collected and qPCR performed. Transcript levels were first normalized to GAPDH as a reference gene, and then to control for comparison to various treatments. All data are mean ± SEM (n=3, treatments and control).

Major Task 2: Determine the specific wnt pathway responsible for prevention of mechanical allodynia in a mouse model of peripheral nerve injury and correlate this with macrophage polarization state and IL-6 to IL-10 ratio.

Summary of Results of Major Task 2: Non-canonical wnt agonists lead to release of anti-inflammatory mediators from macrophages in culture which should reduce chronic sensitization and pain. However, canonical wnt agonists and *not non-canonical agonists, r*educe chronic sensitization (pain) in a mouse peripheral nerve injury model.

Supernatant from the macrophage cultures used in Major Task 1 above were subjected to ELISA for IL-6 and IL-10. The results are shown in Figure 3 below. Results support the findings from Major Task 1 above. The noncanonical wnt ligand (wnt6) shown above to favor M2 macrophage phenotype also leads to increased IL-10 and decreased IL-6 in the culture supernatant. There is no significant change with either wnt3a or the gsk3 inhibitor.

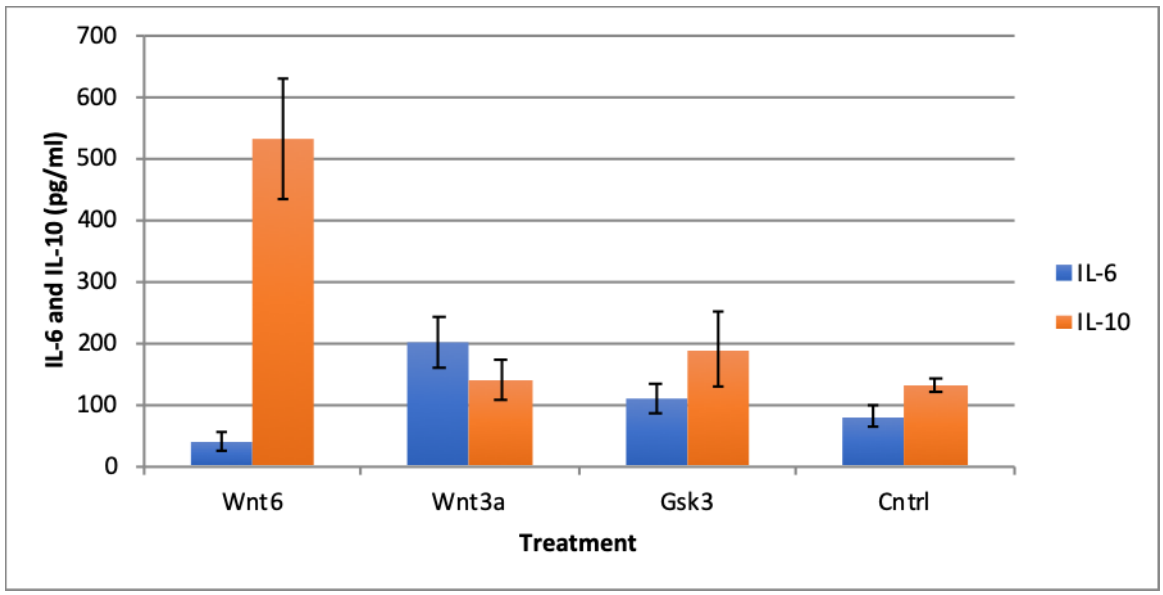

Figure 3) Non-canonical wnt ligand treatment of murine peritoneal macrophages favors expression of the anti-inflammatory cytokine IL-10. Supernatant from murine peritoneal macrophage cultures were collected after treatment with either wnt pathway ligands (wnt6 100ng/ml, wnt3a 100ng/ml, gsk3 inhibitor 100ng/ml or saline as control) or control and ELISA for IL-6 and IL-10 performed. All data are mean ± SEM (n=3, treatments and control).

We treated mice using the spared nerve injury model with intraperitoneal wnt agonists. Our first experiment had three groups: sham, SNI and SNI treated with the canonical wnt agonist wnt3a. The SNI and wnt3a groups both exhibited mechanical allodynia after surgery, but interestingly, by day 15 and 18, the wnt3a group appears to be recovering compared to the SNI group. The number of mice in each group was 5.

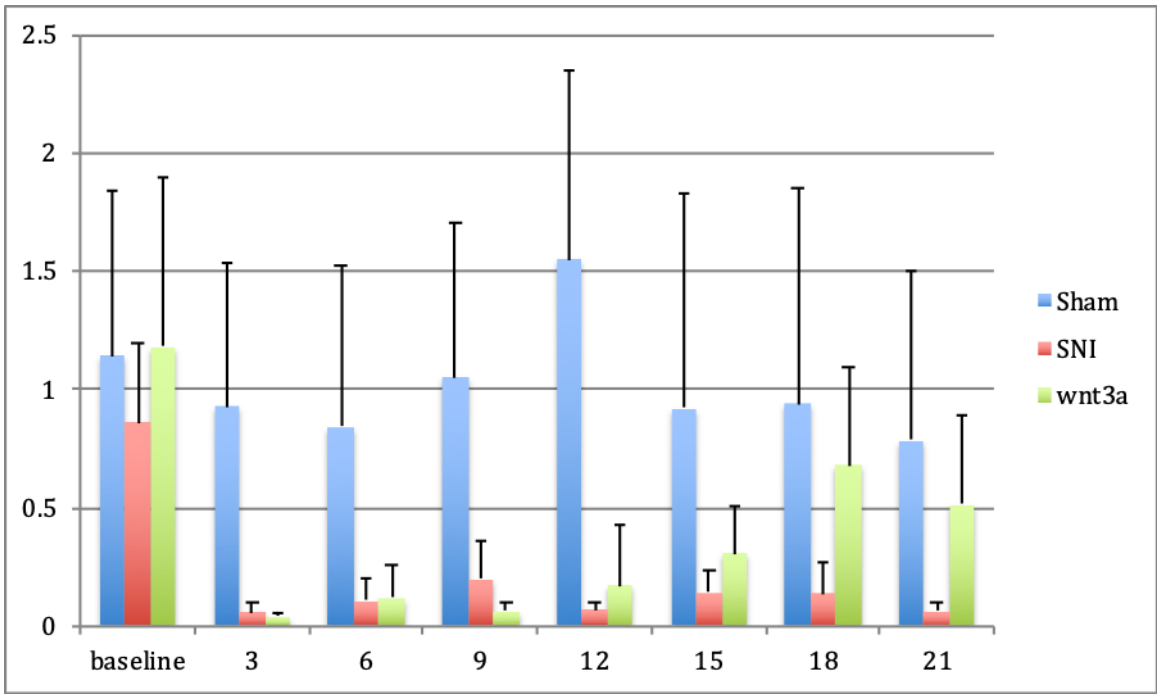

Figure 4: Mice either received sham surgery or SNI surgery. Three groups of 5 animals were tested. Sham animals maintained baseline withdrawal responses, SNI and wnt3a (100ng) treated animals developed significant allodynia with some recovery at the final timepoints. X-axis is time in days and y-axis is force in grams.

Mice treated with wnt3a in the SNI model showed late recovery from allodynia suggesting that wnt3a was able to reverse the late phase of mechanical allodynia development after peripheral nerve injury. We then did the same experiment with the non-canonical wnt agonist wnt5a and found no difference between the SNI group and the treated group suggesting that wnt5a does not play a role in preventing or treating mechanical allodynia (Figure 5 below).

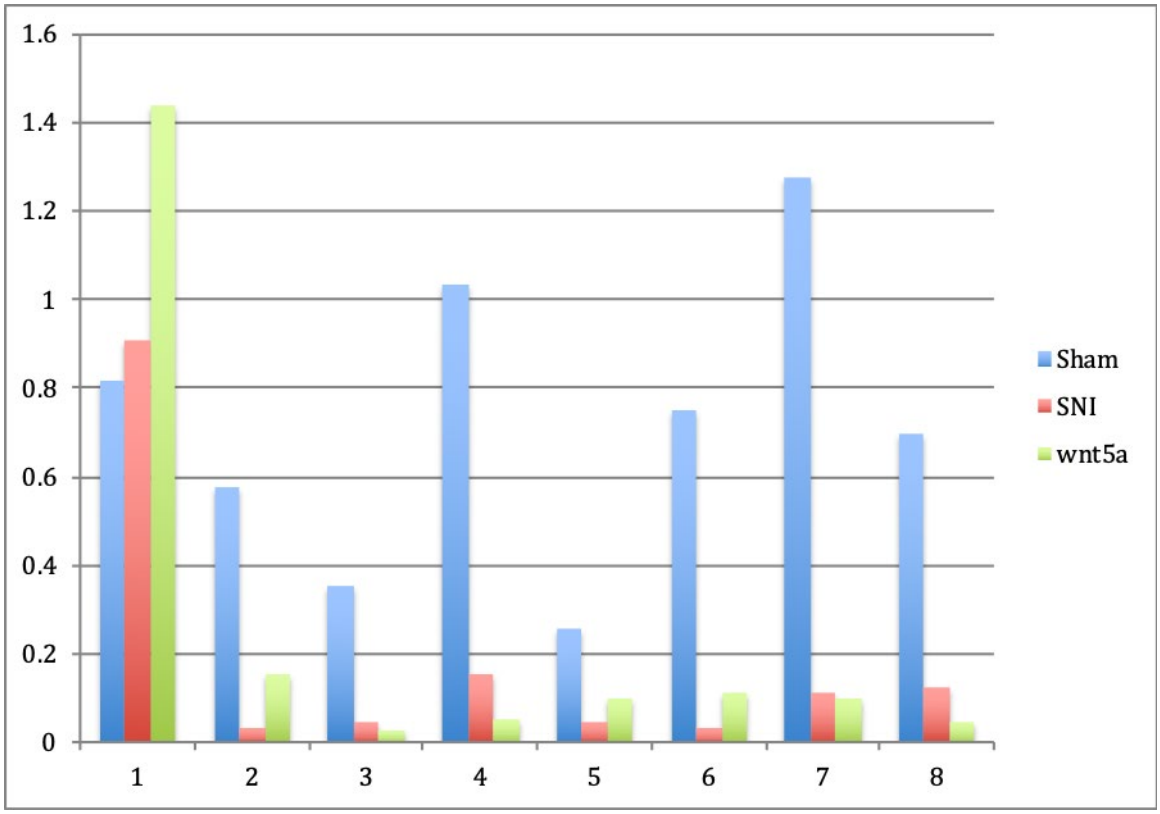

Figure 5: Mice either received sham surgery or SNI surgery. Three groups of 5 animals were tested. Sham animals maintained baseline withdrawal responses except for days , SNI and wnt5a (100ng) treated animals developed significant allodynia with no recovery at the final timepoints. X-axis is time period of 3 days with time period 1 equal to baseline, time period 2 to 3 days post-op and so on. Y-axis is force in grams.

Major Task 3: Characterize wnt pathway expression and DNA methylation changes in humans before and after amputation and determine the role of cytokine ratio measurement in prediction of pain phenotype.

Summary of Results of Major Task 3: Patients with post-amputation pain have increased expression of non-canonical wnt constituents.

This work was done by the lab at UHSHS and the results are displayed in Table 1 below.

Table 1: Expression analysis of selected wnt pathway constituents in patients with and without residual limb pain enrolled in the Veterans Integrated Pain Evaluation Research (VIPER) Valproate study. Test Group is patients with significant chronic pain after amputation and Control group is patients without significant pain.

		AVG ΔC_t (Ct(GOI) - Ave Ct (HKG))		$2^{-\Delta C_t}$		Fold Change	T-TEST
Symbol	Well	Test Group	Control Group	Test Group	Control Group	Test Group /Control Group	p value
AES	A01	1.37	1.37	3.9E-01	3.9E-01	1.00	0.827761
APC	A02	5.22	5.42	2.7E-02	2.3E-02	1.15	0.440798
AXIN1	A03	8.34	8.28	3.1E-03	3.2E-03	0.96	0.915174
AXIN2	A04	7.81	7.77	4.5E-03	4.6E-03	0.97	0.858549
BCL9	A05	7.09	6.81	7.3E-03	8.9E-03	0.82	0.283710
BTRC	A06	4.29	3.89	5.1E-02	6.7E-02	0.76	0.182309

CCND1	A07	11.71	12.25	3.0E-04	2.1E-04	1.45	0.753014
CCND2	A08	4.35	4.53	4.9E-02	4.3E-02	1.14	0.464004
CSNK1A1	A09	2.87	2.99	1.4E-01	1.3E-01	1.09	0.311428
CSNK2A1	A10	3.54	3.52	8.6E-02	8.7E-02	0.99	0.945641
CTBP1	A11	7.81	7.16	4.5E-03	7.0E-03	0.64	0.089098
CTNNB1	A12	5.41	5.71	2.4E-02	1.9E-02	1.23	0.206971
CTNNBIP1	B01	8.76	8.20	2.3E-03	3.4E-03	0.68	0.175266
CXXC4	B02	13.48	12.65	8.7E-05	1.6E-04	0.56	**0.045895**
DAAM1	B03	4.03	3.78	6.1E-02	7.3E-02	0.84	0.162158
DAB2	B04	6.89	6.87	8.4E-03	8.5E-03	0.99	0.681499
DIXDC1	B05	9.57	10.51	1.3E-03	6.9E-04	1.92	0.830047
DKK1	B06	13.25	13.33	1.0E-04	9.7E-05	1.06	0.680486
DKK3	B07	9.48	10.28	1.4E-03	8.0E-04	1.75	**0.037258**
DVL1	B08	11.54	12.06	3.4E-04	2.3E-04	1.43	0.214902
DVL2	B09	7.59	7.20	5.2E-03	6.8E-03	0.76	0.110098
EP300	B10	1.59	1.68	3.3E-01	3.1E-01	1.06	0.810124
FBXW11	B11	2.06	2.28	2.4E-01	2.1E-01	1.16	0.238778
FBXW4	B12	5.64	6.07	2.0E-02	1.5E-02	1.35	0.225165
FGF4	C01	13.34	13.42	9.6E-05	9.1E-05	1.06	0.738745
FOSL1	C02	9.47	11.43	1.4E-03	3.6E-04	**3.88**	0.371707
FOXN1	C03	12.73	12.67	1.5E-04	1.5E-04	0.96	0.780339
FRAT1	C04	1.18	1.35	4.4E-01	3.9E-01	1.12	0.373166
FRZB	C05	13.36	13.34	9.5E-05	9.6E-05	0.99	0.886971
FZD1	C06	8.13	8.47	3.6E-03	2.8E-03	1.27	0.324389
FZD2	C07	11.76	12.14	2.9E-04	2.2E-04	1.30	0.229652
FZD3	C08	10.25	9.99	8.2E-04	9.8E-04	0.84	0.665601
FZD4	C09	10.83	11.19	5.5E-04	4.3E-04	1.29	0.722938
FZD5	C10	12.30	12.16	2.0E-04	2.2E-04	0.91	0.877539
FZD6	C11	6.59	6.99	1.0E-02	7.8E-03	1.32	0.414390
FZD7	C12	12.91	13.08	1.3E-04	1.2E-04	1.12	0.337378
FZD8	D01	11.73	11.66	2.9E-04	3.1E-04	0.96	0.919974
FZD9	D02	13.56	13.41	8.3E-05	9.2E-05	0.90	N/A
GSK3A	D03	3.59	3.54	8.3E-02	8.6E-02	0.96	0.972919
GSK3B	D04	2.17	2.31	2.2E-01	2.0E-01	1.10	0.427464
JUN	D05	9.85	10.38	1.1E-03	7.5E-04	1.44	0.549852
KREMEN1	D06	6.51	7.19	1.1E-02	6.9E-03	1.60	0.201839
LEF1	D07	3.82	3.72	7.1E-02	7.6E-02	0.93	0.324675
LRP5	D08	13.04	12.82	1.2E-04	1.4E-04	0.86	0.904707
LRP6	D09	10.63	11.56	6.3E-04	3.3E-04	1.90	0.227884
MAPK8	D10	3.25	3.33	1.1E-01	1.0E-01	1.06	0.941818
MMP7	D11	13.35	13.34	9.6E-05	9.7E-05	0.99	0.983627
MYC	D12	1.99	1.93	2.5E-01	2.6E-01	0.96	0.548542
NFATC1	E01	5.60	5.67	2.1E-02	2.0E-02	1.04	0.663590
NKD1	E02	11.68	12.43	3.1E-04	1.8E-04	1.68	0.181741
NLK	E03	3.76	3.72	7.4E-02	7.6E-02	0.97	0.878115
PITX2	E04	13.11	N/A	1.1E-04	N/A	**N/A**	N/A

PORCN	E05	5.06	4.86	3.0E-02	3.4E-02	0.87	0.233609
PPARD	E06	4.04	4.04	6.1E-02	6.1E-02	1.00	0.700269
PRICKLE1	E07	9.81	9.02	1.1E-03	1.9E-03	0.58	0.834382
PYGO1	E08	12.33	13.40	1.9E-04	9.2E-05	**2.11**	N/A
RHOA	E09	-1.40	-1.47	2.6E+00	2.8E+00	0.95	0.824762
RHOU	E10	4.89	4.93	3.4E-02	3.3E-02	1.03	0.556089
RUVBL1	E11	5.53	5.61	2.2E-02	2.1E-02	1.06	0.494081
SFRP1	E12	13.03	13.45	1.2E-04	8.9E-05	1.34	0.347165
SFRP4	F01	13.34	13.47	9.6E-05	8.8E-05	1.09	0.585831
SOX17	F02	12.58	12.97	1.6E-04	1.2E-04	1.30	0.343161
TCF7	F03	2.88	2.60	1.4E-01	1.6E-01	0.83	0.410625
TCF7L1	F04	5.42	6.07	2.3E-02	1.5E-02	1.57	**0.029909**
TLE1	F05	5.12	5.54	2.9E-02	2.1E-02	1.34	0.212153
VANGL2	F06	12.75	12.45	1.4E-04	1.8E-04	0.81	0.769881
WIF1	F07	13.21	13.10	1.1E-04	1.1E-04	0.92	N/A
WISP1	F08	12.04	12.56	2.4E-04	1.7E-04	1.43	0.192040
WNT1	F09	12.68	12.95	1.5E-04	1.3E-04	1.20	0.404303
WNT10A	F10	11.31	11.47	3.9E-04	3.5E-04	1.12	0.772472
WNT11	F11	10.14	10.54	8.9E-04	6.7E-04	1.32	0.159065
WNT16	F12	10.48	11.14	7.0E-04	4.4E-04	1.58	0.161718
WNT2	G01	13.46	13.58	8.8E-05	8.2E-05	1.08	0.557335
WNT2B	G02	9.47	8.52	1.4E-03	2.7E-03	0.51	0.122255
WNT3	G03	13.14	13.13	1.1E-04	1.1E-04	0.99	0.996189
WNT3A	G04	12.98	13.10	1.2E-04	1.1E-04	1.09	0.478357
WNT4	G05	8.70	9.86	2.4E-03	1.1E-03	**2.24**	**0.008144**
WNT5A	G06	13.24	13.25	1.0E-04	1.0E-04	1.01	0.979006
WNT5B	G07	12.30	11.84	2.0E-04	2.7E-04	0.73	0.191571
WNT6	G08	12.62	13.20	1.6E-04	1.1E-04	1.50	**0.020234**
WNT7A	G09	8.59	8.57	2.6E-03	2.6E-03	0.99	0.837574
WNT7B	G10	6.29	6.29	1.3E-02	1.3E-02	1.00	0.609665
WNT8A	G11	13.23	13.13	1.0E-04	1.1E-04	0.93	0.630452
WNT9A	G12	13.28	13.36	1.0E-04	9.5E-05	1.06	0.718681
ACTB	H01	-3.41	-3.39	1.1E+01	1.1E+01	1.01	0.741311
B2M	H02	-2.52	-2.62	5.7E+00	6.1E+00	0.94	0.623559
GAPDH	H03	-0.63	-0.26	1.5E+00	1.2E+00	1.29	0.075609
HPRT1	H04	7.50	7.24	5.5E-03	6.6E-03	0.83	0.740671
RPLP0	H05	-0.94	-0.96	1.9E+00	1.9E+00	0.98	0.985785

Our colleagues at USUHS have collected wnt pathway expression data using wnt expression arrays on 22 patient samples - 12 cases (pain greater than 3/10) and 10 controls (pain <3/10) from the VIPER valproate patient cohort. They have discovered 5 wnt pathway constituents with significantly different RNA expression in amputees with pain compared to those without. Interestingly, four of these five genes reduce canonical wnt pathway activity and increase noncanonical activity. The fifth gene is an inhibitor of Disheveled 1 which plays a role in both pathways so it's meaning is unclear at this point. Below is Table 2 showing just the genes with significant changes between case and control along with fold change.

Symbol	Well	Test Group	Control Group	Fold Change	P value
CXXC4	B02	8.7E-05	1.6E-04	0.56	**0.045895**
DKK3	B07	1.4E-03	8.0E-04	1.75	**0.037258**
TCF7L1	F04	2.3E-02	1.5E-02	1.57	**0.029909**
WNT4	G05	2.4E-03	1.1E-03	**2.24**	**0.008144**
WNT6	G08	1.6E-04	1.1E-04	1.50	**0.020234**

Table 2: Wnt pathway constituent gene expression in VIPER Valproic Acid study amputees 3 months after amputation.

1) CXXC4 encodes Idax which is an inhibitor of Disheveled1. Disheveled proteins mediate both canonical and noncanonical wnt pathways so it is difficult to make a conclusion regarding CXXC4 overexpression in the control group since

2) DKK3 is an inhibitor of canonical wnt (Cell *150*, 351–365, July 20, 2012)

3) TCF7L1 is an inhibitor of canonical wnt (Sci Rep. 2016; 6: 28299.)

4) Wnt 4 is a non-canonical wnt ligand and also inhibits canonical signaling (Cell *150*, 351–365, July 20, 2012)

5) Wnt 6 is a non-canonical wnt ligand (Cell *150*, 351–365, July 20, 2012)

Specific Aim 2: Determine the role of TGR5 in astrocyte activation and treatment of mechanical allodynia in a mouse model of neuropathic pain.

Major Task 1: Determine role of TGR5 signaling in astrocyte activation

Summary of Results of Major Task 1: Astrocyte activation is reduced by TGR5 agonism which is important because astrocyte activation in the spinal cord is likely responsible for the development of long lasting neuropathic pain.

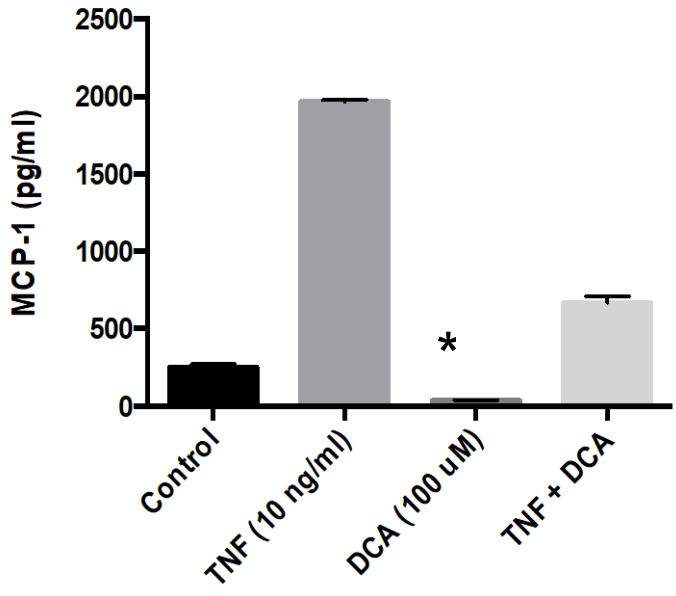

Figure 6: Deoxycholate reduces the release of the chemokine MCP-1 by astrocytes stimulated with TNF *in vitro*. Astrocytes release MCP-1, which contributes to central sensitization and neuropathic pain. Blocking MCP-1 has been shown to attenuate neuropathic pain in mice.

Astrocyte culture work showed a significant decrease in astrocyte activation at the highest dose of the TGR5 agonist (Figure 7 below). This is a very interesting result that strengthens our argument that the longer term, more chronic portion of the transition from acute to chronic pain is modulated by the TGR5 pathway through spinal cord astrocytes.

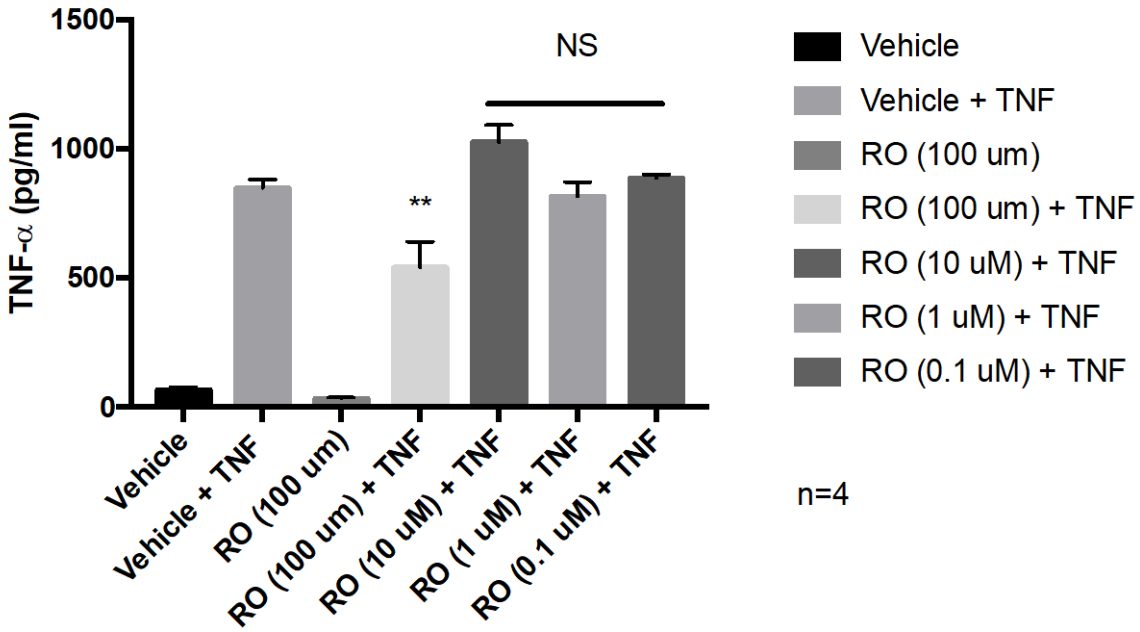

Figure 7: Astrocyte culture activated either by vehicle or TNF while treated with different concentrations of TGR5 agonist (RO)

Major Task 2: Determine the role of TGR5 signaling in treating mechanical allodynia in a mouse peripheral nerve injury model

Summary of Results of Major Task 2: TGR5 agonists reduce already existing mechanical sensitization in mouse models of neuropathic pain.

The TGR5 agonist (deoxycholate) reduces baseline mechanical sensitivity in C57Bl6 mice (Figure 8)

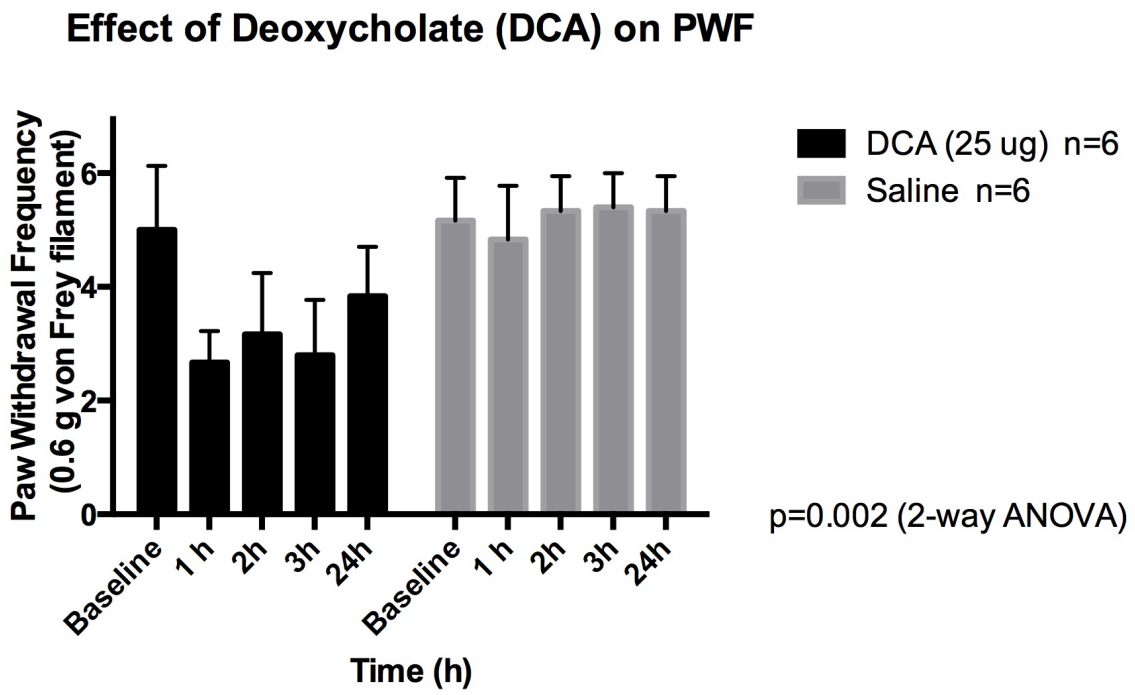

Figure 8: DCA reduces mechanical sensitivity at baseline

We also found that the TGR5 agonist deoxycholate reduces mechanical allodynia in a peripheral nerve injury mouse model most dramatically at 21 days after injury suggesting that the effect is occurring through an astrocyte activation pathway as astrocyte activation occurs in the late stages of neuropathic pain transition (Figure 9)

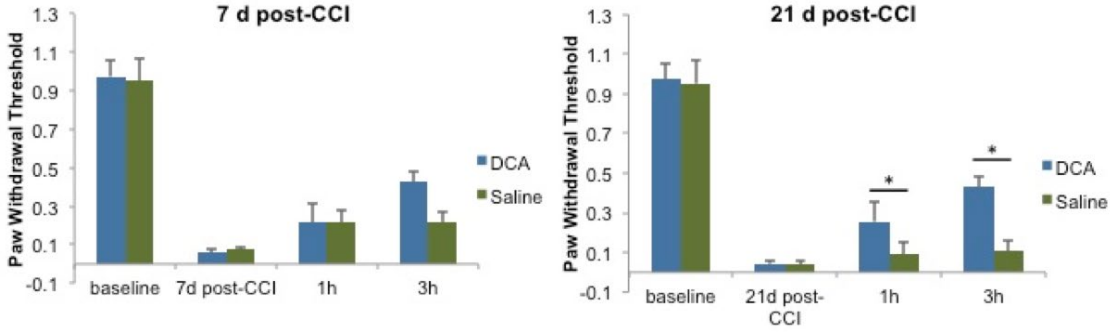

Figure 9: DCA reduces mechanical allodynia in a CCI model of peripheral nerve injury

Most excitingly, the orally available TGR5 agonist obtained from Roche decreases mechanical allodynia after SNI peripheral nerve injury at 7 days after injury (Figure 10)

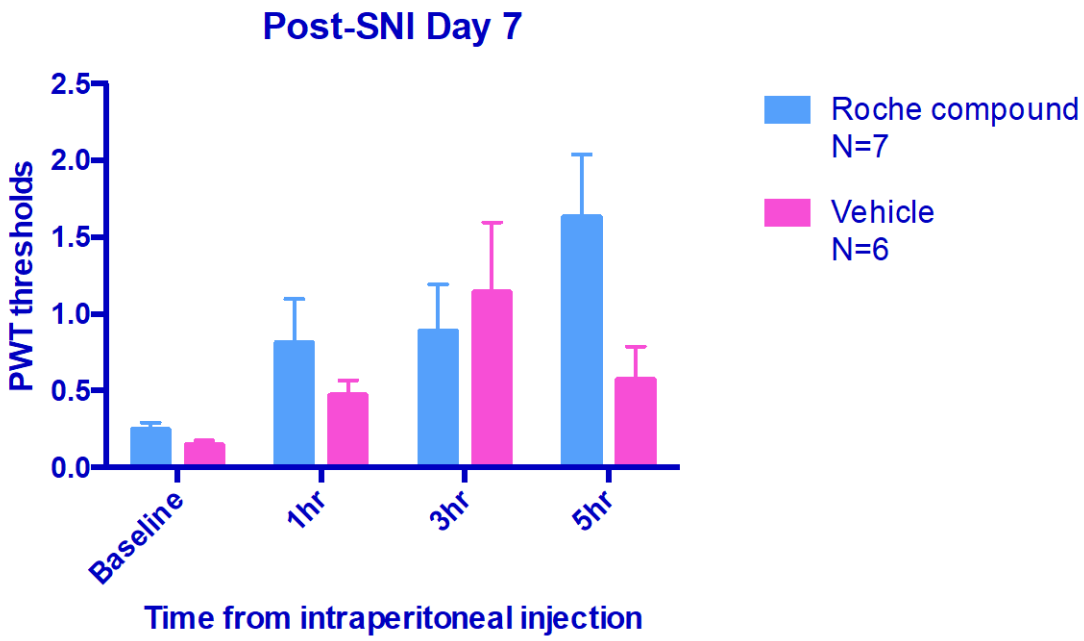

Figure 10: Paw withdrawal threshold is increased (allodynia relieved) by treatment with the Roche TGR5 agonist at day 7 after SNI surgery at the 5hr timepoint.

Specific Aim 3: Use existing data from the Vanderbilt EMR and genotyping repositories to look for associations between genetic variants and pain phenotypes

Major Task 1: Preliminary analyses conducted to confirm the precise numbers of patients for whom there are sufficient data available. Validation of previously published genotype-phenotype associations.

Summary of Results of Major Task 1: Analysis of 30,000 patients from the Vanderbilt DNA databank found 3,654 opioid related adverse events (ORAE) and found associations between 18 genes and ORAE including the adipocyte plasma membrane associated protein gene (*APMAP*) which showed the strongest association. This was confirmed in a larger UK database.

Our colleagues at Vanderbilt extracted data of >30,000 patients from the BioVU DNA databank who were treated with intravenous opioids after a major surgical intervention, and have phenotyped them via links to EMR records with regards to our primary outcome: patients who experienced an inpatient opioid-related ADE (respiratory depression as indexed by naloxone administration) and controls (patients who did not have an ADE). They used a similar case-control phenotyping approach for two secondary outcomes: presence of a rapid response team code and administration of a motility drug (to index constipation). Using the International Classification of Disease, 9th Edition, we identified and pulled information on patient-related risk predictors of opioid-related ADEs. They conducted a preliminary study using existing phenotypes in the dataset potentially relevant to ADEs, in particular, respiratory insufficiency, failure and arrest. They identified 3,654 patients with opioid related adverse reactions in the Vanderbilt BioVu biobank. This search identified 18 distinct genes that showed a statistically significant association with the aggregate of respiratory insufficiency, failure and arrest. Of these genes, adipocyte plasma membrane associated protein gene (*APMAP*), showed the strongest association with a combination of respiratory insufficiency, failure and arrest. Subsequently, using the United Kingdom Biobank, in a set of 500,000 subjects,

they were able to validate the above described association between *APMAP* gene and the occurrence of respiratory insufficiency, failure and arrest.

Major Task 2: Discovery and validation of novel exomic variants associated with opioid adverse drug events.

Summary of Results of Major Task 2: Analysis of 75,000 patients from the Vanderbilt DNA databank using newly available megachip data found 15 single nucleotide polymorphisms associated with opioid related adverse events, 5 of which are present within known genes. These SNPs can either be used as markers of susceptibility to ORAE or can be further studied to discover why they predispose patients to ORAEs.

Our collaborators at Vanderbilt performed the discovery portion of this aim looking at over 770,000 variants in 75,000 patient samples comparing those patients receiving naloxone compared to control and found a number of significant hits including fifteen that survive false discovery rate correction. Below is the workflow for the discovery portion of the project.

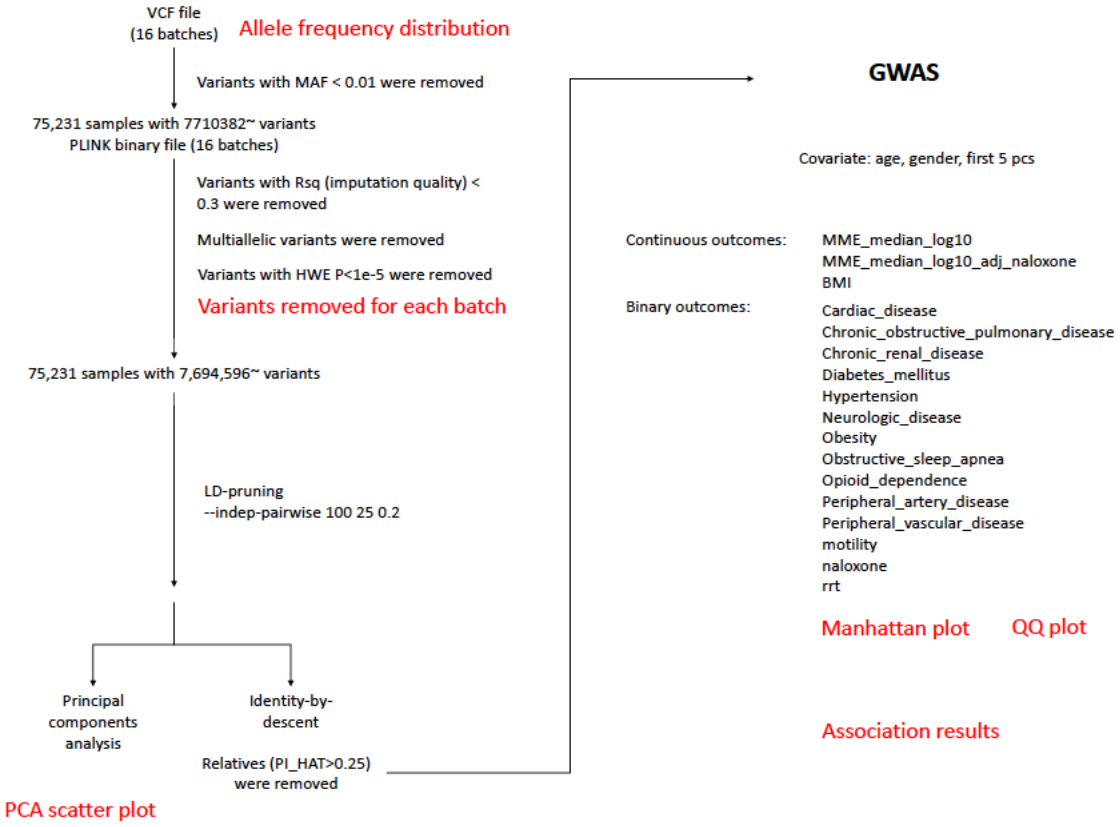

Figure 11: Flowchart of study design showing sample and variant numbers and outcome measures.

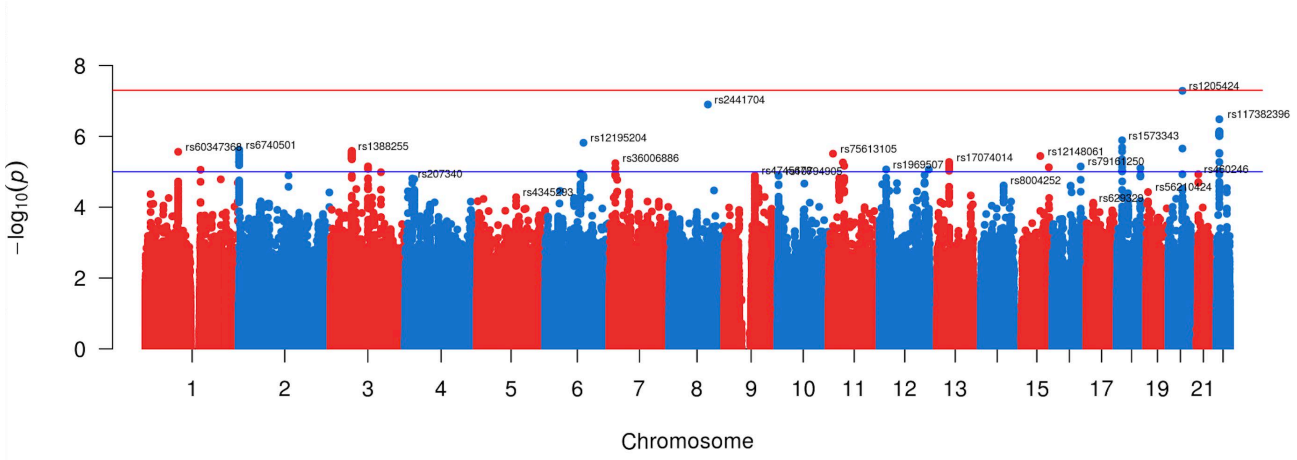

Figure 12: Manhattan plot of variants found to be significantly different between patients receiving naloxone and those not requiring naloxone during hospitalization.

The most significant SNP from each chromosome that also has -$\log_{10}$(p) value of 5 or greater is found in the table below along with the associated gene if the SNP is present in or near a coding region. dbSNP used to lookup individual SNPs.

Table 3 List of SNPs found to be significantly enriched in patients requiring naloxone (A surrogate for presence of opioid related adverse event)

SNP	Location (Chrom)	(-$\log_{10}$(p))	Associated gene (if exonic)
rs60347368	1	5.7	
rs6740501	2	5.8	
rs1388255	3	5.9	ERC2
rs207340	4	4.9	
rs12195204	6	6.0	GRIK2
rs36006886	7	5.4	
rs2441704	8	7.0	
rs75613105	11	5.6	GALNT18
rs1969507	12	5.1	
rs17074014	13	5.2	DLEU1
rs12148061	15	5.4	
rs79161250	16	5.1	
rs1573343	18	6.1	
rs1205424	20	7.3	KIAA1755
rs117382396	22	6.7	

GRIK2 – ionotropic glutamate receptor (kainite receptor) – Involved in EPSC in many regions of the CNS.
ERC2 – cytomatrix protein that regulates neurotransmitter release.
GALNT18 - Polypeptide N-Acetylgalactosaminyltransferase 18 - Important in post-translation modification.
DLEU1 – unknown
KIAA1755 – unknown

The SNPs themselves may be important in predicting patients who are susceptible to opioid related adverse events, but it is interesting to note that two of the SNPs that are exonic are found in genes that affect neurotransmitter release.

IMPACT:
What was the impact on the development of the principal discipline(s) of the project?

Impact of Specific Aim 1: Results of this work showed that non-canonical wnt antagonism is anti-inflammatory in macrophages in vitro, but in animal models of pain, non-canonical wnt antagonism is not analgesic. In fact, canonical wnt agonists seem to prevent chronification of pain in these animal models. Our human data matches the animal data in that patients with pain overexpress non-canonical wnt pathway constituents. This leads our group to believe that overexpression of non-canonical wnt constituents could act as a molecular marker post-amputation pain risk and that therapies that activate canonical wnt over non-canonical may be analgesic.

Impact of Specific Aim 2: TGR5 agonists (either small molecule or bile acid based) reduce astrocyte activation and treat established neuropathic pain in animal models. These results combined with previous human biomarker data collected by our group suggests TGR5 agonism may be a novel analgesic in humans.

Impact of Specific Aim 3: We discovered SNPs in five genes newly associated with opioid related adverse events. We believe these can be used as biomarkers of opioid therapy risk in patients and may provide avenues of future research into why some patients are more susceptible to ORAEs.

What was the impact on society beyond science and technology?
Pain and opioid abuse continue to be costly and deadly problems for service members, veterans and the general public. We believe the results of specific aim 2 and 3 may easily be translated to clinical care to help decrease the burden of pain and opioid abuse.

PRODUCTS:

1. *Published: Chamessian A, Young M, Qadri Y, Berta T, Ji RR, Van de Ven T. Transcriptional Profiling of Somatostatin Interneurons in the Spinal Dorsal Horn. Scientific Reports, 2018 May 1;8(1):6809.*

2. *Published: Alexander Chamessian, Thomas Van de Ven, Thomas Buchheit, Hung-Lun Hsia, Mary McDuffie, Eric Gamazon, Colin Walsh, Stephen Bruehl, Chester 'Trip' Buckenmaier III, Andrew Shaw. Differential Expression of Systemic Inflammatory Mediators in Amputees with Chronic Residual Limb Pain. January 2017 - Volume 158 - Issue 1 - p 68–74.*

3. *Published: Chamessian A, Qadri Y, Cummins M, Berta T, Hendrickson M, Buchheit T, Van de Ven T, "5-hydroxymethylcytosine (5hmC) and Ten-eleven translocation 1-3 (TET1-3) proteins in the dorsal root ganglia: expression and dynamic regulation in neuropathic pain." Somatosensory & Motor Research*

4. *Poster: Bile Acid Signaling TGR5 and Neuropathic Pain. Can Agonism of the G-Protein Coupled TGR5 Receptor Modulate the Development of Chronic Pain? Michele Hendrickson,MD[1], MS Alexander G. Chamessian BS [1,5,6], BS, Alex Kieber BS[1], Hung-Lun Hsia MD[1,3] Thomas Buchheit MD [1,3], Mary McDuffie, RN[2], Chester Buckenmeier MD[2], Andrew Shaw MB FRCA[1,3], Thomas Van de Ven MD PhD[1,3] Duke Academic Evening Symposium April 2017.*

5. *Poster: A method for the isolation and characterization of monocytes from human peripheral whole blood samples. Bronwyn Southwell M.D., Alexander Chamessian B.S., Stuart A Grant MB., ChB., Thomas Van De Ven M.D., Ph.D. Duke Academic Evening Symposium May 2016*

TGR5 manuscript in preparation

Wnt signaling results are currently contradictory and will require further study to determine which pathway might be a therapeutic analgesic option

ORAE manuscript in preparation

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Name: Thomas Van de Ven
Project Role: Principal Investigator
Nearest person month worked: 4.58
Contribution to Project: Coordinates all aspects of the project and assumes overall responsibility for its success.

Name: Ru-Rong Ji
Project Role: Co Investigator
Nearest person month worked: 0.48
Contribution to Project: He is responsible for interpreting and troubleshooting the proposed animal behavioral testing and cell culture experiments and his lab provides deep expertise in all experimental procedures

Name: Sarah Crews
Project Role: Program Manager
Nearest person month worked: 1.80
Contribution to Project: Overall project manager for all aspects of the proposal, including coordination of the biological samples, shipment of samples between sites and data organization, and ensures that the supplies are ordered and available

Name: Thomas Buchheit
Project Role: Co Investigator
Nearest person month worked: 0.24
Contribution to Project: Works closely with Dr. Van de Ven on all aspects of the project
Funding Support: Other resources

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
Organization Name: Vanderbilt University Medical Center
Location of Organization:1161 21st Avenue South, Nashville, TN 37232-2520
Partner's contribution to the project: Collaborated in the research

Organization Name: Henry M. Jackson Foundation for the Advancement of Military Medicine Inc.
Location of Organization: 6720 A Rockledge Drive, Bethesda, MD 20817
Partner's contribution to the project: Collaborated in the research