DOES A FUNCTIONAL SNP VARIANCE IN INTERLEUKIN-6 LEAD TO INCREASED PHYSIOLOGY OF DELIRIUM IN ICU PATIENTS?

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Delirium is an acute alteration in mental status that impairs cognitive and physical functions. It presents with inattention, hallucinations, agitation, and overall cognitive and perceptual disturbances. Although older populations tend to experience delirium more frequently, the incidence of delirium in the intensive care unit (ICU) occurs in up to 80% of the population regardless of age. ICU delirium not only creates acute consequences for patients, but also may negatively affect patient outcomes in the long-term. Studies have associated ICU delirium with longer hospital stay, longer period of mechanical ventilation, increased incidence of cognitive impairment at discharge, and increased mortality risk regardless of age, sex, race, and severity of illness. Nonetheless, current delirium prevention, diagnosis, and treatment are inadequate due to a lack of understanding of its pathology.

This study aimed to explore a relationship between the biomarker interleukin-6 (IL-6) and delirium. Specifically, *IL-6* has a polymorphism at position -174 of C/G that has shown to have effects on IL-6 production in different disease processes. This polymorphism was examined in relation to delirium for a relationship that may help explain delirium's pathogenesis. The first specific aim was to explore if higher frequencies of a specific allele of *IL-6* were present in patients who developed delirium. The second aim was to explore if a specific allele is also associated with more days of delirium. DNA was extracted from blood samples collected by a parent study and analyzed utilizing allelic discrimination in the Applied Biosystems ™ QuantStudio™ 3 Real-Time PCR System.

Data analysis included descriptive statistics, Pearson chi-square tests, univariate analyses, t-tests comparing genotype and days of delirium experienced, and a binary logistic regression model. These tests were utilized to find any association between *IL-6* -174 genotypes (alleles C/G) with delirium and delirium positive days. We did not find statistically significant conclusions to support a specific genotype as being associated with delirium and delirium positive days. Additional studies that explore biomarkers in the role of delirium are needed to further investigate the biology and pathology of delirium to promote the development of proper prevention, diagnosis, and treatment in ICU patients and improve patient outcomes.

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1.0 INTRODUCTION

In the intensive care unit (ICU), patients' bodies undergo extreme stress due to critical illness and life-saving medicine. One complication of the physical and psychologic stress, exacerbated by the disruptive environment, is delirium. Delirium is an acute change in mental status that impairs cognitive and physical function presenting as the inability to focus, visual hallucinations, and agitation.[2] Particularly in the ICU, delirium is sometimes expressed as a decrease in level of consciousness and decreased interaction with the environment. Studies have found that delirium is associated with longer hospital stay, longer period of mechanical ventilation, higher incidence of cognitive impairment at discharge and up to one year later, and increased mortality regardless of the severity of illness, age, gender, and race. [1,2,21] Additionally, the cost of hospital stay is substantially greater for those who experience delirium, and potentially even 20% greater if it were not for the association of early ICU mortality.[3] While delirium commonly presents in hospitalized or post-operative older adults, delirium rates are higher in ICU patients. In the ICU, the incidence of delirium is up to 80% of patients and inflicts people of all ages.[4]

Even with these significant and poor outcomes, delirium is underdiagnosed and therefore inadequately managed.[2] Tools such as the Confusion Assessment Method (CAM) and a specialized Confusion Assessment Method for Intensive Care Unit (CAM-ICU) are utilized by doctors and nurses at the bedside to determine the presence and monitor the severity of delirium in patients.[5] There are barriers decreasing the usability of the CAM-ICU.[6] Nurses reported that the CAM-ICU

was more of a complicated task added to their already extensive workload, rather than a beneficial tool that supplements a routine physical assessment of the patient.[6] Researchers noted that the utilization of the CAM-ICU had lower performance when completed by the bedside nurses, but high sensitivity and specificity when completed by research nurses.[8] Another disadvantage to the CAM-ICU is that it requires the patient to be experiencing delirium symptoms at the time of assessment. The Intensive Care Delirium Screening Checklist (ICDSC) is a delirium monitoring tool that evaluates patient behaviors over a period of 8-24 hours for delirium symptoms: level of consciousness, inattention, disorientation, hallucinations/delusions/psychosis, psychomotor agitation or retardation, inappropriate speech or mood, sleep/wake cycle disturbances, and symptom fluctuation.[22] An important difference between the CAM-ICU and the ICDSC exists in the timing of the exam. The CAM-ICU assesses and scores a patient only at the time of the exam, whereas the ICDSC accounts for temporal symptom fluctuation over a longer time frame when calculating the final score.[23] Regardless of the tool used, discrepancy between assessments can easily occur with the lack of objective material, especially in the mechanically ventilated patient population. Additional limitations to these tools include potential for recall bias and knowledge discrepancies when implementing. While these tools are both fairly easy to use and have good reliability and validity, it has been proposed that delirium may be missed 36% of the time with the ICDSC and 38% of the time with the CAM-ICU.[29] With serious implications associated with delirium, it is imperative to optimize assessment tools' sensitivity to delirium detection.

van den Boogaard et al.[8] completed the first delirium prediction study in ICU patients. Their PREdiction of DELIRium for Intensive Care patients (PRE-DELIRIC) model includes 10 weighted risk factors that are accessible within 24 hours after intensive care admission: age, APACHE-II score, admission category, coma, infection, metabolic acidosis, use of sedatives and

morphine, urea concentration, and urgent admission.[8] The PRE-DELIRIC model had excellent predictive ability – a 0.87 area under the receiver operating characteristics curve with a 95% confidence interval 0.85 to 0.89.[8] It has good validity compared to intensive care nurses' and physicians' assessments, (N=124).[8] Ultimately, the PRE-DELIRIC model proved superior (0.87; 0.81-0.93) in predicting delirium compared to the nurses (0.59, 0.49 to 0.70) and physicians (0.59, 0.49 to 0.70).[8] The model has been used in six different countries for ICU patients with similar results.[10] This powerful tool would be able to help providers implement early intervention and prevent delirium in patients, which is crucial in improving patient outcomes.[10] Nonetheless, the PRE-DELIRIC model has not been implemented worldwide, most likely due to its limitations including a lack of incorporation of temporal changes in physiologic state over the ICU admission.[8] In the ICU, patients can experience rapid changes in their health status in short periods of time, and with these changes the risk of the development of delirium will change as well. Implementation of the PRE-DELIRIC model into clinical care requires development of a computer program to query admission medical records, or dedicated staff, to identify risk with follow up communication to staff. Despite these tools for predicting and assessing for developing delirium, they are not routinely utilized in all ICU's, meaning a considerable proportion of delirium is not identified or treated. A biomarker, genetic or otherwise, capable of predicting delirium risk would greatly enhance the ability to provide improved individualized surveillance, prevention, and possible treatment, thereby decreasing negative sequelae of ICU delirium.

Nevertheless, even when delirium or delirium risk is identified early, preventative methods or interventional management yields poor results. Haloperidol, the standard typical antipsychotic used for delirium, causes extrapyramidal adverse effects in patients and has recently been found to not be effective in decreasing delirium symptomology.[30] Other atypical antipsychotics such as

risperidone, olanzapine, or quetiapine do not improve outcomes in patients who develop ICU delirium.[14] Benzodiazepines have also not been helpful to control non-alcohol related delirium.[12] However, one study found that dexmedetomidine, a selective alpha-2 adrenergic receptor agonist, was associated with more delirium-free and coma- free days compared to lorazepam in mechanically ventilated ICU patients.[12] Non-pharmacologic interventions for delirium address risk factors of delirium such as sleep deprivation and sensory impairment. One study focused on intervention protocols utilized for at-risk older adults, and compared the rate of occurrence and duration of delirium in older adults receiving usual care. These protocols covered a wide range of activities: orientation protocols included boards with dates and names; therapeutic activities protocol used cognitively stimulating activities three times a day with word games or discussion of current events; non-pharmacologic sleep protocol at bedtime utilized warm drinks and relaxation music; sleep enhancement protocol worked on noise reduction in the environment and readjusting schedules around sleeping; early mobilization protocol required participants to exercise three times a day and minimize immobilization equipment such as restraints and catheters; vision protocol reinforced participants to use glasses, magnifying glasses, and introduced more large-print books and telephone keypads; hearing protocol encouraged the usage of special communication techniques and amplifying devices; and the dehydration protocol encouraged oral intake of fluid.[13] Incorporating this extensive environmental and treatment modification protocol did decrease the incidence of delirium (9.9% vs. 15%) and total number of days of delirium (105 days vs. 161 days) in older adults. However, results were dependent on consistent utilization of the labor-intensive interventions which is challenging and often not feasible in non-research settings. The intervention was significantly less effective once delirium developed, reinforcing that the most important treatment of delirium is prevention.[13] Applying these tactics in a hospital's ICU

poses another set of obstacles due to physiologic or hemodynamic instability and limited time for providers to administer these tools. While behavioral interventions and environmental modification decreases delirium incidence somewhat, it remains a persistent problem.

Identification of individuals at risk of delirium, and preventative or treatment strategies targeting pathology would improve delirium care and patient outcomes. To date, there is not a clear understanding of related pathology to provide efficient, focused interventional treatment. Furthermore, the biology driving ICU delirium symptoms is unknown, hampering advances in evidence-based care towards reducing the burden of delirium on ICU patients. There are countless widely accepted predisposing risk factors for ICU delirium, but many critical patients who do not develop delirium have these risk factors, limiting specificity. Risk factors include advanced age, hypertension, diabetes, previous psychiatric history, hypoxia, hypothermia, prolonged mechanical ventilation, and disrupted sleep patterns.[11] Without a clearer mechanism to identify patients who are at a higher risk for developing delirium, patients are at risk to be under-diagnosed and insufficiently treated.

Current research proposes ICU delirium is a consequence of sustained systemic inflammation. Apolipoprotein E (APOE) genotype, a gene with functional polymorphisms, has been associated with neuro-inflammation and delirium in elderly medical inpatients both in and out of the ICU.[15,33] There has been conflicting evidence for a role of APOE in delirium as others have found no association, and a recent meta-analysis found no relationship.[16,34] Individuals who were admitted with a hip fracture were found to have an association between delirium and increased levels of C-reactive protein and soluble *IL-6* receptor.[24] Other work has supported an association between IL-6, a pro-inflammatory cytokine, and delirium in ICU patients. Alexander et al. measured daily serum levels of specific biomarkers in ICU patients experiencing delirium.[16]

They found that there is an association between higher serum levels of IL-6 in ICU patients with delirium.[16] Pre-operative IL-6 levels were also found to be significantly increased in a post-operative delirium population.[17] With this confirmation in the relationship between IL-6 and delirium, it is important to pursue the source of variability of this important biomarker.

Important factors to consider with delirium are the mechanisms of the underlying and ongoing pathology that brought the patient to the ICU in the first place. Having an infection or systemic inflammatory response syndrome (SIRS) where there is inflammation throughout the body may change the pathway of delirium than if it were occurring in a patient without an inflammatory pathology. Specific pro-inflammatory and anti-inflammatory biomarkers were studied in patients with and without delirium and with or without infection or SIRS. Researchers found that the pro-inflammatory cytokine IL-8 was significantly associated with delirium in inflamed patients while anti-inflammatory cytokine IL-10 was associated with delirium in patients without inflammation.[25]

Studying the gene and its polymorphisms can lead researchers into understanding more of the relationship between IL-6 and delirium. In its promoter region, *IL-6* has a common single nucleotide polymorphism (SNP) of C and G at position -174, or rs1800795. There are conflicting reports of *IL-6* -174C/G genotype, and specifically presence of the C allele, having protective characteristics across multiple conditions. Poor health outcomes have been associated with a -174GG expression, such as lipid abnormalities and increased risk of intracranial hemorrhage, whereas the -174CC expression has shown a potential to have protective factors against systemic-onset juvenile chronic arthritis (S-JCA) and Eales' disease (ED).[18,19,20,26] Research suggests that the *IL-6* -174C/G polymorphism expression correlates with serum IL-6 concentration and disease processes; however, this has not been studied in the context of delirium or critical care.

In active S-JCA, a pro-inflammatory condition, patients experience systemic adverse effects such as high fevers, lymphadenopathy, hepatosplenomegaly, myalgia, arthritis, and increased amounts of inflammatory biomarker C-reactive protein (CRP).[19] In addition to CRP, researchers found in this population *IL-6* -174CC homozygotes had a decreased presence of plasma IL-6 than GG homozygotes and GC heterozygotes.[19] The variation in the presence of these inflammatory biomarkers in association with the polymorphism is significant in its influence of developing S-JCA although other genetic or environmental triggers likely contribute to the development of this disease process. Sen et al. found that elevated IL-6 serum concentrations were associated with the inflammatory stage of Eale's disease (ED), or an idiopathic inflammatory vasoproliferative disease of the retina.[26] Polymorphisms at the position of -174 on *IL-6*, specifically the -174GG genotype, were found to be functionally significant with increased IL-6 serum concentrations in patients with ED. The -174CG genotype was higher in their control group and found to have a protective factor against ED, suggesting the C allele has a protective response and can mask the effects of the G allele in heterozygotes.[26] Another study found the -174CC genotype increased serum IL-6 levels, increasing gastrointestinal involvement in systemic sclerosis.[27] Although a discrepancy over protective genotypes exists, all of the disease processes linked with *IL-6* -174C/G polymorphisms have inflammatory pathways and mechanisms involved. Given the literature showing ICU delirium is a pro-inflammatory state, it is important to further analyze genetic variability of *IL-6* in the context of delirium and if the -174C/G polymorphism is a protective or predisposing factor. Based on its association with IL-6 protein variability, pro-inflammatory diseases and other poor health outcomes, *IL-6* -174C/G has the potential in playing an important role in the inflammatory pathway that results in delirium.

Delirium has been described as organ failure of the brain.[4] Nonetheless, the biopathology driving delirium symptoms remain poorly understood.[7] Given the high level of variability in delirium, and particularly ICU delirium development with few specific predictors, it is important to consider exploring genetic variability in the context of delirium. Analyzing biomarkers that may predispose patients to delirium would help expand our knowledge and inform future work aimed at personalized medicinal approaches to improve patient outcomes. By exploring genetic factors that place patients at higher risk for delirium, the consequences of delirium can be diminished or avoided.

2.0 PURPOSE OF STUDY

The purpose of this study was to explore a potential relationship between delirium and *IL-6* -174 C/G polymorphism. The specific aims of this study were to 1. determine if a specific genetic polymorphism has a higher frequency in individuals who developed delirium, and 2. to determine if it is associated with more days of delirium experienced.

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This study has a prospective, observational design. It utilized targeted allelic discrimination to determine *IL-6* -174C/G genotype. The use of de-identified samples and participant's demographic data, collected for a parent study (PI- S. Alexander), was approved by the University of Pittsburgh's Institutional Review Board (STUDY18100068).

3.2 PARTICIPANTS

Participants in this study were originally recruited for the parent study which focused on genetic variability in inflammatory biomarkers and delirium. These participants were specifically studied for associations between delirium presence, duration, and outcomes in ICU patients with protein-based inflammatory markers (IL-6, IL-8, and IL-10), APOE protein, and related genetic variability.

Participants were recruited from a 24 bed Medical ICU and a 22 bed Surgical-Trauma ICU. After informed consent was obtained from the patient or proxy, demographic data including sex, age, race, history of inflammation/sepsis/infectious process, current inflammation/sepsis/infection process, anti-inflammatory or infection medications, was extracted from the medical records. Research staff assessed for delirium every morning for the first 5-10 days with the CAM-ICU. Days of delirium, presence of an acute brain injury (ABI; delirium or

coma), days of an ABI, number of days free of delirium, and number of days free from ABI were collected.

Inclusion criteria for participants included age ≥18 years old, admission to ICU and on mechanical ventilation for greater than 24 hours, and no known preadmission cognitive disorder. Adults were the main focus of the population since children have a different ICU delirium frequency and recovery, likely due to biological differences. The aim was to recruit individuals at highest risk for ICU delirium, so ensuring ICU admission and mechanical ventilation for greater than 24 hours targets that population. Cognitive disorders may bias delirium assessment and interpretation of results. Patients with current or pre-existing neurologic diseases or deficits, including alcohol withdrawal, mild cognitive impairment, dementia, hypoxia/anoxia, previous stroke or head injury, were excluded. Additional exclusion criteria included not having a proxy available to provide consent or being unable to consent themselves, and having a positive toxicology screen in which drugs or withdrawal from drugs may confound delirium results.

Participants were Caucasian or African American, ranging from 18-85 years of age. The study protocol changed during the data collection period such that the first 77 participants had delirium data collected up to 5 days while 58 had delirium data collected for 10 days.

3.3 DELIRIUM ASSESSMENT

Delirium assessment was performed daily (10 days) to ensure capture of the ICU delirium development. To determine an accurate presence of delirium in participants, the Richmond Agitation Sedation Scale (RASS) was performed daily as a precursor to the CAM-ICU to measure sedation levels. Subjects with RASS scores rating between -3 to +4 underwent delirium assessment

using the CAM-ICU, while the remaining patients (RASS -4 or -5) or those who were not sedated but unresponsive, were designated as being in a comatose state (whether disease or drug induced). Participants were rated as either normal (CAM-ICU negative), delirious (CAM-ICU positive and RASS -3 or higher) or comatose (RASS -4 or -5). Those who were categorized as comatose or delirious were further grouped into an acute brain dysfunction positive group, since both are states of acute cognitive dysfunction in ICU patients.

The CAM-ICU was selected due to its ease of use, brevity, established reliability and validity, and frequent use enabling comparisons across studies. Raters were trained and tested to determine interrater reliability for the RASS and CAM-ICU by separately assessing patients, blinded to each other's results, and continued training until 90% agreement was reached.

3.4 DNA EXTRACTION AND QUANTIFICATION

DNA was extracted from blood samples taken from existing arterial or central venous catheters daily (day 1-10) throughout the study. One 3mL sample and one 5mL sample was taken from each participant for the parent study. Samples were stored in a refrigerator in a research laboratory in the clinical site until transfer to the molecular genetics lab at the University of Pittsburgh's School of Nursing adjacent to the clinical site. DNA was extracted using a simple salting out technique and stored at 4°C until analyzed in batch.[32]

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3.5 GENETIC DATA COLLECTION

Allelic discrimination of the samples was conducted using an Applied Biosystems ™ QuantStudio™ 3 Real-Time PCR System. To prepare for data collection, a master mix was created in a separate reservoir that contained 12.5µl Taqman Universal PCR Master Mix No AmpErase, 0.625µl 40X concentration assay of rs1800795, and 10µl sterile water. This master mix was pipetted into a MicroAmp Optical 96-Well Reaction Plate with 23µl in each well. Then, 2µl of extracted DNA was then introduced into the well and centrifuged at 1500rpm to ensure all materials amalgamated. Once all of the wells had the master mix and DNA, an optical adhesive film covered the tops of the well plate to enhance fluorescent light for the PCR system to read. Each allele had a specific fluorescent symbol: The C allele was VIC specific on the X- axis and the G allele was FAM specific on the Y-axis.

Table 1. Master Mix Preparation

Sterile water	10µl
40X Concentration Assay (rs1800795)	0.625µl
TaqMan Universal PCR Master Mix No AmpErase	12.5µl
Extracted DNA	2µl
	25µl per sample

The cycling conditions of the QuantStudio™ 3 Real-Time PCR System from Applied Biosystems™ required 5 steps of Holding, PCR, and Post-read. The holding stage maintained 95°C for 10 minutes. The plates then entered the PCR stage which includes steps 2-4. Step 2 held the plates at 95°C for 15 seconds, and moved onto step 3 where the plates were reduced to 58°C for 1 minute and 30 seconds. Step 4 cycled steps 2 and 3 again 50 times. The post-read stage held

the plates at 60°C for 30 seconds. In total, the allelic discrimination was completed in 135 minutes for each plate. Any results that were inconclusive were excluded from further analysis.

Table 2. Cycling Conditions

	Temperature	Time	Number of cycles
Stage 1	95°C	10 minutes	1 cycle
Stage 2	95°C	15 seconds	1 cycle
Stage 3	58°C	1.5 minutes	1 cycle
Stage 4	Repeat Stage 2 and 3		50 cycles
Stage 5	60°C	30 seconds	1 cycle

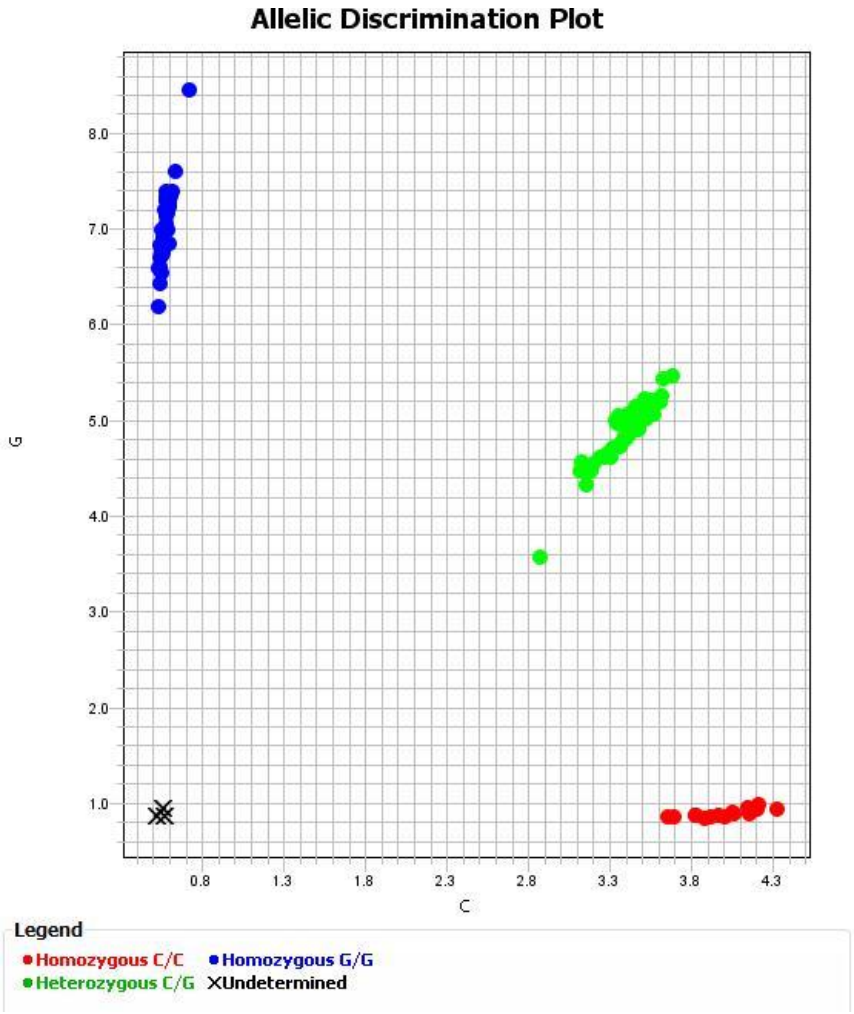

Figure 1. PCR System Genotype Plot

3.6 STATISTICAL ANALYSIS

SPSS Statistics Version 24.0 Software (IBM Corp., Armonk, New York) was used to run statistical analyses on the collected data.

3.6.1 Preliminary data analysis

Absolute frequency and percentage were reported for all categorical variables while measures of central tendency and dispersion were obtained for continuous variables.

Differences in the dependent variable of the presence of delirium subjects with different genotype (independent variable) were analyzed with a Pearson Chi-Square test. Categorical demographic data was also analyzed by delirium to identify any potential covariates: age, sex, race, history of inflammatory disease, acute process of inflammatory disease, and use of anti-inflammatory medications. To further explore any relationship, logistic regression test was also completed on these variables.

Demographic data grouped by genotypes was examined with chi-square and ANOVA tests, and then examined with t-tests for delirium positive days.

3.6.2 Analysis of specific aims

Specific Aim 1. Determine if a specific genetic polymorphism has a higher frequency in individuals who developed delirium. To address specific aim 1, frequencies and percentages of *IL-6* genotypes, Chi-Square tests, and t-tests were completed with the dependent variable of the presence of delirium. Genotype was also analyzed with other independent variables to assess for

the presence of confounding variables. A univariate logistic regression test was completed on the presence of delirium as well.

Specific Aim 2. Determine if this polymorphism is associated with more days of delirium experienced. To address specific aim 2, delirium days within each different genotype group was analyzed utilizing a t-test.

4.0 RESULTS

The sample was comprised of 134 patients with clinical data and samples. The average age of the sample was 52.5 years old who were predominantly female (n = 75), Caucasian (n = 121), and had the heterozygous genotype CG (n = 61). Although, genotype variation was similar between GG (n = 55) and CG (n = 61). Additional demographic data collected on the patients are included in table 3.

Table 3. Sample Demographics

Age (years)	
Mean (Standard Deviation)	52.46 (17.23)
Range (Min, Max)	67 (18, 85)
Sex	
Male	59 (44%)
Female	75 (56%)
Race	
Caucasian	121 (90.3%)
Black/African American	13 (9.7%)
Other	0 (0%)
Genotype	
GG	55 (41%)
CG	61 (45.5%)
CC	18 (13.4%)
History of inflammation/sepsis/infection	
No	109 (81.3%)
Yes	25 (18.7%)
Acute inflammation/sepsis/infection	
No	110 (82.1%)
Yes	24 (17.9%)
Anti-inflammatory/infection medications	
No	103 (76.9%)
Yes	31 (23.1%)
Delirium Presence	
No	64 (47.8%)
Yes	70 (52.2%)

Delirium Days	
Mean (Standard Deviation)	2.83 (1.93)
Median	2.00

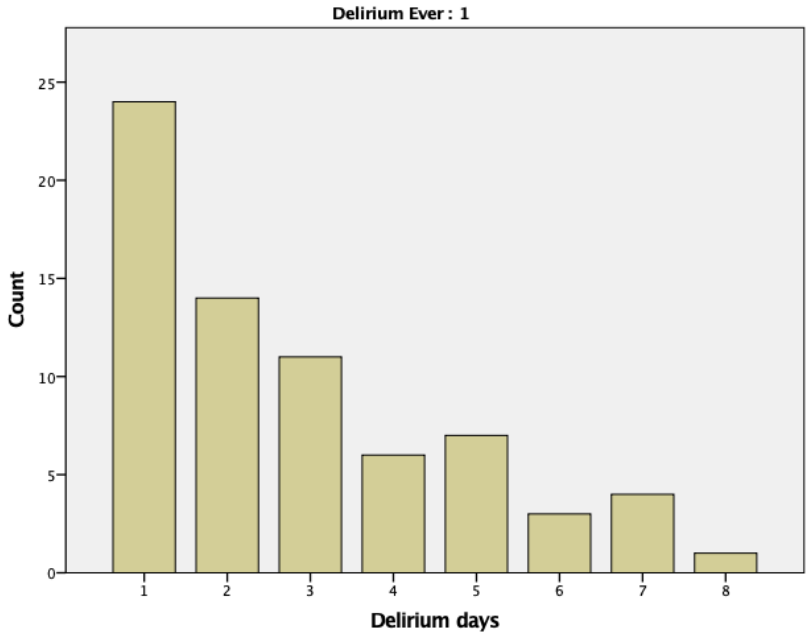

Figure 2. Delirium Duration

Acute respiratory failure and acute respiratory distress were the most common admitting diagnoses. About 64 patients (47.8%) never experienced delirium throughout the study while 70 patients (52.2%) experienced delirium positive at least one day in the study. For the delirium positive sample, the average days of delirium experienced was 2.83 days (standard deviation [SD] 1.93), with a median of 2 days. Figure 2 shows the unequal, skewed distribution of delirium positive days that patients experienced.

Patients were recruited from the University of Pittsburgh's Medical Center Presbyterian's Medical ICU and Surgical-Trauma ICU. The assessment tools are standardized between the two different units, and both utilized the RASS and CAM–ICU to diagnosis delirium. Neither unit provided consistent, standardized delirium preventative measures for the patients, so the usage of

lights, reorientation, and administration of antipsychotics/sedatives for patients can be ignored for the purpose of this study due to lack of fidelity.

The sample was grouped together by their respective genotypes to further understand the demographic distribution as reported in table 4 utilizing chi-square tests and an ANOVA test for age. Differences in genotype presentation are especially important when considering the different races present within the sample and the implications of allele frequencies in different races. There was a significant association between genotype and race ($p = 0.018$) and genotype and anti-inflammatory/infection medications ($p = 0.018$).

Table 5 presents the t-test results of analyzing effects of genotype on the amount of delirium positive days. Genotype was grouped as either G-positive, encompassing groups GG and CG compared to CC, or C-positive which encompassed groups CG and CC compared to GG.

Table 4. Demographic Data by Genotype

	GG (n=55)	**CG (n=61)**	**CC (n=18)**	**p-Value**
Age (years)				0.455
Minimum	18	18	19	
Maximum	79	85	82	
Average (Standard Deviation)	54.3 (16.5)	52.0 (17.8)	48.5 (17.7)	
Sex				0.997
Male	24 (40.7%)	27 (45.8%)	8 (13.6%)	
Female	31 (41.3%)	34 (45.3%)	10 (13.3%)	
Race				0.018
Caucasian	45 (37.2%)	58 (47.9%)	18 (14.9%)	
Black/African American	10 (76.9%)	3 (23.1%)	0 (0.0%)	
Other	0 (0%)	0 (0%)	0 (0%)	

History of inflammatory, sepsis, or infectious process				0.339
Yes	7 (28%)	14 (56%)	4 (16%)	
No	48 (44%)	47 (43.1%)	14 (12.8%)	
Acute inflammatory, sepsis, or infectious pathway				0.677
Yes	8 (33.3%)	12 (50%)	4 (16.7%)	
No	47 (42.7%)	49 (44.5%)	14 (12.7%)	
Anti-inflammatory and infection medications				0.018
Yes	6 (19.4%)	20 (64.5%)	5 (16.2%)	
No	49 (47.6%)	41 (39.8%)	13 (12.6%)	
Presence of delirium				0.768
Yes	29 (41.4%)	33 (47.1%)	8 (11.4%)	
No	26 (40.6%)	28 (43.8%)	10 (15.6%)	

Table 5. Genotype and Delirium Positive Days

	t-value (p-value)	**Mean difference (95% CI)**
C-Positive	-0.754 (0.453)	-0.355 (-1.294, 0.584)
G-Positive	0.508 (0.613)	0.371 (-1.086, 1.828)

Delirium distribution among the sample the demographic data was analyzed utilizing univariate analyses as reported in table 6. Pearson Chi-Square tests were completed on categorical variables to assess any relationship with delirium as displayed in table 7. Sex and delirium did not have a significant relationship ($p = 0.268$), race did not have a significant relationship with delirium

(p = 0.903), and genotype did not have a significant relationship as well (p = 0.768). Age is known risk factor for developing delirium, but was not found to have a significant association in this sample's presence of delirium (p = 0.565).

Table 6. Demographic Data by Delirium

	Delirium Negative (n=64)	Delirium Positive (n=70)	Total (N=134)	p-Value
Age (years)				0.565
Minimum	19	18		
Maximum	85	82		
Average (Standard Deviation)	51.6 (16.6)	53.3 (17.8)		
Sex				0.268
Male	25 (42.4%)	34 (57.6%)	59 (100%)	
Female	39 (52%)	36 (48%)	75 (100%)	
Race				0.903
Caucasian	58 (47.9%)	63 (52.1%)	121 (100%)	
Black/African American	6 (46.1%)	7 (53.8%)	13 (100%)	
Other	0 (0%)	0 (0%)	0 (0%)	
Genotype				0.768
GG	26 (47.3%)	29 (52.7%)	55 (100%)	
CG	28 (45.9%)	33 (54.1%)	61 (100%)	
CC	10 (55.6%)	8 (44.4%)	18 (100%)	
History of inflammation, sepsis, infectious process				0.426
Yes	17 (42.5%)	23 (57.5%)	40 (100%)	
No	47 (50%)	47 (50%)	94 (100%)	
Acute inflammation,				0.227

sepsis, infectious process				
Yes	15 (39.5%)	23 (60.5%)	38 (100%)	
No	49 (51%)	47 (49%)	96 (100%)	
Anti-inflammatory, infection medications				0.925
Yes	26 (47.3%)	29 (52.7%)	55 (100%)	
No	38 (48.1%)	41 (51.9%)	79 (100%)	

Genotype and delirium were further analyzed utilizing a logistic regression test to control for potential explanatory variables of age, sex, race, history of inflammation/sepsis/infection, acute inflammatory/sepsis/infectious process, and anti-inflammatory/infection medications. The Hosmer and Lemeshow Test showed that the data did not conflict ($p = 0.283$) with any assumptions made by the binary logistic regression model. Even with all variables controlled for, none of the clinical data showed significant association with delirium as shown in table 7.

Table 7. Binary Logistic Regression Test

	OR (95% CI)	**p-Value**
Age	0.987, 1.029	0.458
Sex (Male)	0.774, 3.220	0.209
Race (Caucasian)	0.327, 3.654	0.885
Genotype		0.724
GG	0.389, 3.727	0.747
CG	0.506, 4.425	0.466
History of inflammation, sepsis, infection	0.291, 2.327	0.713
Acute inflammatory, sepsis, infectious process	0.416, 2.661	0.915
Anti-inflammatory, infection med	0.762, 5.187	0.160

5.0 DISCUSSION

Increased levels of pro-inflammatory cytokine IL-6 have been found in ICU patients who develop delirium and those with worse outcomes.[16] Further exploration of IL-6 and delirium's relationship could help provide insight into understanding delirium's mechanisms. A functional polymorphism in *IL-6* leads to genotypic variability in the production and expression of IL-6 serum concentration. In pro-inflammatory diseases, *IL-6* -174 C/G polymorphism has been found to be a key determinant. Specifically, the minor allele C has been shown to be protective against the inflammatory diseases generating lower IL-6 concentration, while the G allele has been associated with increased serum concentrations of IL-6 in participants with active inflammatory diseases.[18.19,20,26] Therefore, pursuing a study of this specific polymorphism, *IL-6* -174 C/G, was a logical step in the exploration of the source of individual and genetic variability in delirium.

We did not find the *IL-6* -174 C/G polymorphism significantly associated with delirium presentation in patients nor the amount of delirium positive days. We utilized the binary logistic regression in an attempt to control for other variables, but still found no relationship. Other work has found the CC genotype of the *IL-6* -174 genotype is protective against pro-inflammatory diseases, so the binary logistic regression model utilized this genotype as the reference group. Nonetheless, discrepancies between which allele had protective factors exists.[27] This discrepancy was taken into account when analyzing the data as separated into either a C positive or G positive group. If there is one allele that has more influence on the gene expression, then separating into these groups would have accounted for it. Genotype and race was found to have a statistically significant association, as well as genotype and anti-inflammatory/infection medications.

Ancestral background influences our genetic makeup and the allelic frequencies of this polymorphism are different between races so this significant association was to be expected. Genotype and use of anti-inflammatory or infection medications were also found to have a statistically significant relationship. While this may be statistically significant, it may not be clinically relevant or significant. If we were analyzing medications and genotype in a different aspect, such as response to medications based on genotype, this would have been a significant finding. However, this variable was included as a potential covariate in delirium presentation, and these medications were not statistically significant within a chi-square analysis. Even further, when controlled for in a binary logistic regression test, anti-inflammatory/infection medications and delirium did not have a significant association. This relationship may also be a consequence of the small sample size and inadvertently calculating artifact as significant.

Demographic information on patients such as history of inflammation/sepsis/infectious process, acute inflammation/sepsis/infectious process, and anti-inflammatory/infection medications, were included in the analysis to evaluate as potential confounding variables. Since delirium's mechanisms involve the inflammatory pathway, a patient with a known history of inflammatory diseases, such as sepsis or other infection, would be more susceptible to having increased inflammatory biomarkers and risk to develop delirium. Likewise, this would also be true for a patient with an acute inflammatory process during their ICU admission. Additionally, it was important to this study to control take into account for any medications the participant may have been taking that would suppress inflammatory pathways. Despite control for a number of these potentially influential demographic and clinical variables, we did not find statistical significance. There was an uneven distribution between the three different genotypes within the sample. The C allele is the minor allele globally at a frequency of 0.258, while the G allele has a frequency of

0.741. In addition, these allele frequencies differ among races: the European population has a C allele frequency of 0.460 and a G allele frequency of 0.539, the African population's C allele has a frequency of 0.075 and the G allele 0.925.[35]

In this study, the sample contained heavily uneven groups of Caucasian and Black/African American participants. To further investigate allele frequencies within the sample, the Hardy-Weinberg formula was applied and shown to be in equilibrium. From this population, the Caucasian group had a C allele frequency of 0.388 and a G allele frequency of 0.611. The Black/African American group had a C allele frequency of 0.115 and a G allele frequency of 0.885. This genotype distribution shows that this study had an overall under-representation of the C allele in Caucasians and G allele in African-Americans, while there was an overrepresentation of the G allele in Caucasians and C allele in African-Americans. This may have biased our results; however, the small number of minorities makes this unlikely.

While delirium is a pro-inflammatory state with increased serum IL-6 levels noted in ICU patients, it does not appear that the *IL-6* -174C/G allele is driving the variable pathology. There are other factors that may influence *IL-6* expression. Epigenetic mechanisms that can modify transcription can complicate gene expression. For example, higher methylation of the *IL-6* gene or other genetic variability may contribute to the condition. Likewise, upstream regulators like NF-kappa B play important roles in activation of *IL-6*.[31] Genetic variability in other pro-inflammatory genes may contribute to the increased *IL-6* expression as well and may be an informative line of future work.

5.1 LIMITATIONS

Limitations of this study included the sample size, uneven distribution within the sample, and confounding variables. With a small sample and attempting to understand potential protective factors of the minor allele expression, it is difficult to obtain even sample sizes within each genotype group. Nonetheless, having an even number of genotypes within each group would be ideal to see the association between the differing polymorphisms and delirium.

Similarly, it is difficult to control comorbidities and effects of the environment on patients that may affect delirium outcome and presentation. Objective screening tools for delirium also limit consistency and reliability between users who administer the CAM-ICU. Working with a critically-ill population also limits who can be included in studies due to inability to provide consent or unforeseen future complications.

5.2 CONCLUSION

This study was unable to discover a significant relationship between the *IL-6* -174 C/G polymorphism and its impact on delirium and days of delirium presentation. Nonetheless, delirium's pathophysiology is still widely unknown but is proposed to be a consequence of sustained systemic inflammation. With research showing evidence that varying levels of inflammatory markers and proteins are associated with delirium, future studies should continue to pursue this route. Future studies should include larger sample size, equal distribution of genotypes, control for comorbidities, and control for more potential confounding variables, to ensure validity and reliability. Clinical implications of this study point to the future utilization of genetic

biomarkers as a tool to assess a patient's risk or likelihood of developing a disease. Continuing to assess biomarkers that influence the pathophysiology of delirium may be helpful for delirium prevention, detection, and effective personalized treatment in those who are high risk to improve patient outcomes.

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