

Short Report

Association between thiopurine medication exposure and Alzheimer's disease among a cohort of patients with inflammatory bowel disease

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Abstract

Introduction: Ras-related C3 botulinum toxin substrate 1 (Rac1), a member of the Rho-GTPase family of proteins, could be an Alzheimer's disease (AD) triggering co-factor due to its effect on both amyloid precursor protein (APP) and tau. Thiopurine medications, such as azathioprine and mercaptopurine, are immunosuppressants that suppress Rac1 activation. We hypothesize that due to their ability to suppress Rac1, thiopurines are associated with a lower risk of AD.

Methods: To explore the relationship between thiopurines and incident AD diagnosis, we conducted a national retrospective cohort study among U.S. Veterans with inflammatory bowel disease (IBD), including Crohn's disease (CD) or ulcerative colitis (UC), as well as a non-IBD control. We created propensity score-matched cohorts and estimated the hazard ratio via the time-dependent Cox proportional hazards model.

Results: The study sample size was 66,312 patients and consisted of 24,057 IBD patients (4354 thiopurine exposed and 19,703 unexposed) and 42,255 patients without IBD or thiopurine exposure. Patients exposed to thiopurines have the lowest rate of AD, and our results demonstrate for each additional year of thiopurine exposure risk of AD is reduced by 8.3%% (adjusted HR = 0.917; 95% CI = [0.851–0.989]).

Discussion: Our results support the preclinical findings implicating Rac1 in the AD disease process. A national cohort study demonstrated that Rac1 is associated with the AD process consistent with the preclinical evidence. Further exploration and evaluation of Rac1 inhibition are needed.

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Concept and design: All authors. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: All Authors. Statistical analysis: JM. Supervision: SS JH.

Conflict of Interest Disclosures: None.

Funding/Support: None

This paper represents original research conducted using data from the Department of Veterans Affairs. This material is the result of work supported with resources and the use of facilities at the Dorn Research Institute, WJB Dorn Veterans Affairs Medical Center, Columbia, South Carolina. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

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1. Introduction

Thiopurines are immunosuppressants used in inflammatory bowel disease for conditions, such as Crohn's disease (CD) and ulcerative colitis (UC). The thiopurines (azathioprine and mercaptopurine) are also potent inhibitors of Ras-related C3 botulinum toxin substrate 1 (Rac1), a member of the Rho-GTPases family of proteins [1–4]. Rac1 has been implicated in Alzheimer's disease (AD) process [5], leading to increased amyloid precursor protein (APP) processing and stimulated tau hyperphosphorylation [6]. Evidence suggests Rac1 deregulation may be an AD trigger due to its effect on both APP

and tau [6]. Furthermore, Rac1 inhibition in vascular endothelial cells could confer neuroprotection through the upregulation of artemin, a neurotrophic factor [7]. Given the preclinical evidence, we hypothesize that pharmacologic inhibition of Rac1 is associated with lower AD incidence. To test this hypothesis, we examine the association between thiopurines, which inhibit Rac1, and incident AD diagnosis among a national cohort of United States Veterans.

2. Methods

2.1. Data

This national retrospective cohort study evaluating the association between thiopurines and incident AD among IBD patients was conducted using data from the Department of Veteran Affairs (VA). The study period was January 1, 2000, and May 1, 2019, and the data were extracted from the VA Informatics and Computing Infrastructure (VINCI), which includes inpatient, outpatient data (coded with International classification of diseases (ICD) revision 9-CM, revision 10-CM) and pharmacy claims. The completeness, utility, accuracy, validity, and access methods are described on the VA website, <http://www.virec.research.va.gov>. The study was conducted in compliance with the Department of Veterans Affairs requirements, received VA Institutional Review Board, and VA Research & Development approval. The institutional review board of the WJB Dorn Veterans Affairs Medical Center approved a HIPPA waiver for informed consent.

2.2. Cohort creation

We evaluated a cohort of United States Veterans diagnosed with inflammatory bowel disease (IBD), including patients with ulcerative colitis (UC) or Crohn's disease (CD) between January 2000 and April 30, 2018. We further utilized a cohort of patients without IBD and not exposed to thiopurines. Patients were followed from index, identified as the first UC or CD diagnosis date, to the earliest date of (a) Alzheimer's diagnosis (b) death or (c) May 1, 2019. Patients were included if they were (1) age 55 or older at index, (2) had at least one year between index date and study end, (3) had no thiopurine use prior to index and (4) had at least 6 months VA eligibility prior to index. IBD thiopurine exposed patients were matched 1 to 5 to IBD patients with no exposure to thiopurine using greedy nearest neighbor propensity score matching. Additionally, a non-IBD/nonthiopurine exposure cohort was created utilizing the same follow up and inclusion criteria. The index date for the non-IBD cohort was set as 6 months after the VA enrollment date. Each IBD thiopurine exposed case was propensity score-matched using greedy nearest neighbor matching with up to 10 non-IBD controls.

2.3. Outcome and exposure coding

The primary outcome is time to initial diagnosis of AD. AD was coded using ICD-9-CM and ICD-10-CM codes 331.0 and G30.x. Thiopurine use was captured as a time-dependent cumulative exposure with each additional days' supply incrementing the cumulative annual exposure. Thiopurine medications were extracted from the pharmacy files using the generic drug name search.

2.4. Statistical analysis

Baseline characteristics were compared between the three cohorts using the ANOVA F-test for continuous variables and the chi-square test for categorical variables. To analyze incident AD, we calculate the unadjusted incidence rate of AD between the two cohorts. We estimated hazard ratios for any thiopurine exposure via the Cox proportional hazards model and for each additional year of thiopurine exposure via the time-dependent Cox proportional hazards model. The adjusted model includes the comorbidities and medications listed in [Table 1](#).

3. Results

A total of 66,312 patients met study criteria and consisted of 24,057 IBD patients and 42,255 non-IBD/nonthiopurine patients. Among the IBD cohort, 4354 patients were exposed to thiopurines, and 19,703 patients were not exposed to thiopurines. [Table 1](#) summarizes the characteristics of the three cohorts. Because of the large sample size, there are several baseline characteristics that were statistically different. For example, the mean age was statistically different; however, the actual mean age values in years were 66.03, 66.7, and 66.04. Baseline characteristics that were clinically and statistically significant include: (1) patients exposed to thiopurines have a higher rate of methotrexate and oral corticosteroid use; (2) patients exposed to thiopurines have a longer duration of follow up; and (3) select comorbidities had different rates. [Table 2](#) presents the rates of incident AD, as well as the hazard ratios from the time-dependent Cox model. Thiopurine use was associated with both a lower frequency of patients with AD (1.7%) and a lower rate per 1000 patient-years (1.83) compared to both the non-thiopurine IBD exposed cohort and non-IBD cohort ([Table 2](#)). The Cox proportional hazards model, reveals that thiopurine exposed IBD patients have a 29% lower overall risk of AD compared to non-thiopurine exposed patients (HR = 0.71; 95% CI = [0.55–0.90]) ([Table 2](#)). The non-IBD cohort has a 47% higher overall risk of AD compared to the non-thiopurine exposed IBD patients (HR = 1.47; 95% CI = [1.30–1.66]) ([Table 2](#)). Time-dependent Cox models reveal per each additional year of thiopurine exposure the risk of AD is reduced by 8.6% (HR = 0.914; 95% CI = [0.848–0.985]) in the unadjusted model and 8.3% (HR = 0.917; 95% CI = [0.851–0.989]) in the adjusted model ([Table 2](#)).

Table 1
Baseline characteristics*

Variable	IBD thiopurine exposed N = 4354	IBD unexposed N = 19,703	Non-IBD control N = 42,255	P value
Race				
Black	291 (6.68%)	1445 (7.33%)	2664 (6.305%)	<.001
Other/Unknown	427 (9.81%)	2046 (10.38%)	4137 (9.791%)	
White	3636 (83.51%)	16,212 (82.28%)	35,454 (83.905%)	
Age	66.03 (7.87)	66.7 (8.03)	66.04 (7.897)	<.001
Index year	2006.91 (5.17)	2007.33 (5.76)	2006.61 (4.94)	<.001
Days in study	3533.21 (1821.66)	3188.43 (1900.81)	2620.42 (1616.67)	<.001
Charlson comorbidity	0.97 (1.38)	1.04 (1.34)	1.03 (1.54)	.016
BMI				
<18.5	31 (0.71%)	141 (0.72%)	307 (0.73%)	.763
18.5–24.9	922 (21.18%)	4205 (21.34%)	9025 (21.36%)	.763
25–29.9	1819 (41.78%)	8232 (41.78%)	17,553 (41.54%)	.763
30+	1563 (35.89%)	7021 (35.63%)	15,197 (35.96%)	.763
Missing	19 (0.44%)	104 (0.53%)	173 (0.41%)	.763
Methotrexate	293 (6.73%)	759 (3.85%)	368 (0.87%)	<.001
Oral corticosteroids [†]	3056 (70.19%)	13,203 (67.01%)	9397 (22.2%)	<.001
Pure hypercholesterolemia	344 (7.90%)	1654 (8.39%)	2442 (5.78%)	<.001
Hypertriglyceridemia	249 (5.72%)	1197 (6.08%)	1482 (3.51%)	<.001
Hyperlipidemia	1579 (36.27%)	7508 (38.11%)	11,621 (27.50%)	<.001
Ischemic heart disease	909 (20.88%)	4339 (22.02%)	8792 (20.81%)	.002
Other heart disease	596 (13.69%)	2869 (14.56%)	5745 (13.59%)	.005
Hypertension	2230 (51.22%)	10,387 (52.72%)	20,106 (47.58%)	<.001
Type 2 diabetes	907 (20.83%)	4173 (21.18%)	9319 (22.05%)	.017
Cerebral infarction	19 (0.44%)	105 (0.53%)	286 (0.67%)	.03
Atrial fibrillation	158 (3.63%)	794 (4.03%)	1870 (4.43%)	.008
Hypothyroidism	212 (4.87%)	974 (4.94%)	1601 (3.78%)	<.001
Hyperthyroidism	18 (0.41%)	83 (0.42%)	91 (0.22%)	<.001
Depression	536 (12.31%)	2506 (12.72%)	4125 (9.76%)	<.001
Traumatic brain injury	7 (0.16%)	37 (0.188%)	72 (0.17%)	.867
Alcohol dependence	113 (2.59%)	551 (2.79%)	1701 (4.03%)	<.001
Parkinson's disease	19 (0.44%)	89 (0.45%)	498 (1.18%)	<.001
Generalized anxiety disorder	67 (1.54%)	302 (1.53%)	370 (0.87%)	<.001
Chronic kidney disease	114 (2.62%)	561 (2.85%)	1245 (2.95%)	.418

*Values are number (percentage) unless noted otherwise.

[†]Oral corticosteroids include prednisone, dexamethasone, prednisolone, methylprednisolone, hydrocortisone, budesonide.

4. Discussion

Regulation of Rac1 is a key determinant of neurologic health and is implicated in several neurologic diseases,

including AD [5–11]. Given the wealth of preclinical evidence and the scarcity of human studies of Rac1 and AD, we sought to evaluate the association between Rac1 inhibitors, thiopurines, and incident AD

Table 2
Frequency, rate, and hazard of Alzheimer's disease

Statistic	Cohort		
	Thiopurine exposed	Thiopurine unexposed	Non-IBD control
Frequency and rate of AD			
Frequency: N (%)	77 (1.7%)	446 (2.2%)	1166 (2.7%)
Rate per 1000 patient-years: Rate (95% CI)	1.83 (1.46–2.29)	2.59 (2.36–2.85)	3.85 (3.63–4.07)
Hazard of AD			
Overall hazard of AD: HR (95% CI)*	Thiopurine versus IBD, Thiopurine unexposed Non-IBD control versus Thiopurine unexposed	0.71 (0.55–0.90) 1.47 (1.30–1.66)	
Hazard Per Additional Year			
Time-dependent cox proportional Hazards model: HR Per additional year of thiopurine exposure (95% CI)	Unadjusted Adjusted*	0.914 (0.848–0.985) 0.917 (0.851–0.989)	

*Model is adjusted for comorbidities and medications listed in Table 1. In our time-dependent model, the HR (95% CI) for any methotrexate exposure is 0.69 (0.457–1.048) and for any oral corticosteroid exposure is 0.67 (0.59–0.76).

diagnosis. Utilizing a matched national cohort of patients with and without IBD, our analyses determined that each additional year of exposure to thiopurines is associated with an 8.3% lower risk of developing AD. Consistent with preclinical research, our results suggest Rac1 could represent a therapeutic target in the prevention of AD and should be further investigated. The pathway regarding AD is complex and multifactorial. AD has hallmark characterizations described by abnormal depositions of hyperphosphorylated tau protein in the form of neurofibrillary tangles and of Beta-amyloid peptide in the form of senile plaques. However, the synaptic and dendritic loss may be the best predictor of clinical AD symptoms [12–17]. Therefore, Rac1 could impact the Rho-GTPase signaling deregulation contributing to the synaptic degeneration observed in AD. Specifically, Rac1 is connected to APP processing; however, studies examining Amyloid beta (A β) and Rac1 are discordant [18,19]. Given our sample size and methodology, we believe the data are robust in evaluating Rac1 targets (through thiopurine exposure) and AD. However, there are many factors that may have influenced the outcomes that were not accounted for in this study. Specifically, this study has limitations common to observational claims database analysis that may affect the generalizability to other patient populations. This is a retrospective study of veteran patients comprising mostly of men. Therefore, the results may not be generalizable to non-veterans or to women. Classification error is a limitation of all research utilizing administrative claims. Specifically, ICD9/10 coding was evaluated to define an outcome of a condition by the coding of an observation. It is possible the coding error could have been captured, and this could have led to inaccurate data for diagnosis. While we utilized regression adjustment to explore the association between thiopurines and AD, treatment was not randomized, and we cannot rule out unobserved confounding. Furthermore, medication usage was measured from filled prescriptions, and we cannot confirm patients adhered to the prescriptions [20]. For minimizing bias, our methodology utilized regression adjustment and propensity score matching to account for observed differences in the cohorts. Given the limitations of our study, we feel that our data support the preclinical finding that Rac1 is involved in AD. Therefore, our results, along with preclinical studies, reveal Rac1 could be a viable therapeutic agent for AD warranting further investigation.

Acknowledgments

Author Contributions: JM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read the journal's authorship agreement.

RESEARCH IN CONTEXT

1. Systematic review: Our review of the literature found (a) preclinical papers linking Rac1 to Alzheimer's disease and (b) literature on the ability of thiopurines to suppress Rac1 activation. However, we found no previous paper that linked thiopurines and Alzheimer's disease risk.
2. Interpretation: Our propensity score-matched cohort study found that exposure to thiopurines was associated with a decreased risk of Alzheimer's disease. Our result supports the preclinical finding that links Rac1 and Alzheimer's disease.
3. Future directions: Future studies should (1) further examine the link between pharmacologic inhibition of Rac1 and Alzheimer's disease, and (2) prospective studies examining the thiopurines and Alzheimer's disease.

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