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Review Article

The role of the NLRP3 inflammasome in atherosclerotic disease: Systematic review and meta-analysis

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SUMMARY

Atherosclerosis is a chronic, progressive cardiovascular disease characterized by cholesterol deposition within blood vessel walls. Recent literature has suggested that the NLRP3 [NOD (nucleotide oligomerization domain)-, LRR (leucine-rich repeat)-, and PYD (pyrin domain)-containing protein 3] inflammasome is a key mediator in the development, progression, and destabilization of atherosclerotic plaques. This review aims to evaluate the current literature on the role of NLRP3 in human atherosclerosis.

This systematic review was registered on the PROSPERO database (ID = CRD42022340039) and involved the search of a total of 8 databases. Records were screened in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. A total of 20 studies were included and quality assessed using the NIH: NHLBI tool. Six were eligible for meta-analysis using RevMan 5.4.1.

We identified 20 relevant articles representing 3388 participants. NLRP3 mRNA levels and downstream cytokines, interleukin (IL)-1β and IL-18 were found to be associated with atherosclerotic disease. Fold changes in NLRP3 mRNA levels were most strongly associated with high risk atherosclerotic disease, compared to controls [0.84 (95 % CI: 0.41–1.28)]. IL-1β mRNA fold change was more robustly associated with high-risk atherosclerotic disease [0.61 (95 % CI: 0.10–1.13)] than IL-18 [0.47 (95 % CI: 0.02–0.91)].

NLRP3, IL-1β, and IL-18 are associated with high-risk atherosclerotic disease. However, given the scope of this review, the role of this inflammasome and its cytokine counterparts in acting as prognosticators of coronary artery disease severity is unclear. Several upstream activators such as cholesterol crystals are involved in the canonical or non-canonical activation of the NLRP3 inflammasome and its downstream cytokines. These findings highlight the necessity for further research to delineate the exact mechanisms of NLRP3 inflammasome activation and potential drug targets.

Introduction

Atherosclerosis is a chronic and progressive inflammatory cardiovascular disease (CVD) of high prevalence globally [1]. In 2015, an estimated 422.7 million people were affected by CVD, contributing to 18 million or almost 32 % of all deaths [2]. Atherosclerotic CVD also carries a large financial burden, evidenced by the $126 billion expenditure it caused to the US healthcare system in 2015, which was predicted to increase 3-fold to almost $310 billion by 2035 [3]. Financial burden aside, atherosclerotic disease markedly affects patients' lives [4]. This impact is measured as disability-adjusted life years (DALYs). In 2021, an equivalent of 129 million DALYs were lost due to coronary artery disease (CAD) in the USA [4]. Thus, the burden of atherosclerotic disease on both patients and the health system globally is unequivocal.

As early as the 1980s, studies reported the presence of leucocytes within atherosclerotic arteries, implicating the role of inflammation in the pathophysiology of atherosclerosis [5]. Dagenais and colleagues built on this literature describing inflammasomes as immunity related protein complexes and their likely role in various inflammatory pathology, including atherosclerosis [6]. In 2010, Latz et al. were the first to demonstrate the involvement of inflammasomes, specifically NLRP3, in the pathophysiology of atherosclerosis [7].

NLRP3 is a complex consisting of a pyrin domain-containing protein 3 (PYD), leucine-rich repeat (LRR) and a nucleotide-binding and oligomerization domain (NOD; NACHT) [6–11]. NLRP3 can be activated by a two-signal process [12]. The first signal involves toll-like-receptors and tumor-necrosis-factor (TNF) receptors being stimulated, leading to the activation of nuclear factor κ-B (NF-κB) which increases the

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expression of NLRP3, pro-interleukin (IL)-1β and pro-IL-18. The second signal involves pathogen-associated-molecular-patterns (PAMPs) and damage-associated-molecular-patterns (DAMPs) initiating the assembly of NLRP3, associated-speck-like-protein (ASC), and pro-caspase-1 [12]. Once activated, NLRP3 cleaves pro-caspase-1 to caspase-1 allowing it to cleave pro-IL-1β and pro-IL-18 to their active forms; permitting inflammatory effects to take place. This activation pathway is termed the "canonical pathway" [13].

Throughout the literature, various PAMPs and DAMPs have been implicated in NLRP3 activation. Karasawa et al. suggested that cholesterol crystals (CC) and oscillatory shear stress are involved in the activation of NLRP3 [14]. The role of CC in the canonical pathway via lysosomal damage, upregulation of CD36, and maturation of the atherogenic cytokines IL-1β and IL-18 have also been previously discussed [10,14,15].

The aforementioned pathway is separate to the "non-canonical pathway". Previous literature suggests upstream regulators of this pathway include cytosolic liposaccharide (LPS) from Gram-negative bacteria and other lipids such as oxidized phospholipids, namely oxPAPC [16]. Both LPS and oxPAPC through distinct mechanisms, result in IL-1β release via caspase-11 in murine studies, and caspase-4 and 5 in human studies [11,16,17]. Activation of IL-1β is key in mediating a pro-inflammatory microenvironment via endothelial dysfunction, plaque destablization and other mechanisms [11,17,18]. The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) study further validated the significance of IL-1β by highlighting the therapeutic potential of its selective inhibition with canakinumab [11,19]. This study revealed that canakinumab reduced recurrent cardiovascular events, independent of lipid levels, emphasizing the role of inflammasome-related cytokines in atherosclerosis and its complications [19].

While these findings contribute to elucidating atherosclerosis pathogenesis, current understanding of such pathways is limited by genetically modified ApoE−/− and LDLr−/− murine models. Thus, this review focuses on studies that utilize a primary human model to demonstrate the role of NLRP3 in atherosclerotic pathology. Our objective was to discuss the role of upstream activators, downstream cytokines, the utility of NLRP3 expression as a prognosticator for CAD severity and, its therapeutic potential when inhibited. To the best of our knowledge, our systematic review and meta-analysis is unique in summarizing this.

Methods

We conducted this systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The review was registered on the PROSPERO database (PROSPERO ID = CRD42022340039).

Search strategy

On April 9, 2022, a comprehensive database search was performed including studies published on OVID Medline and Web of Science (Web of Science Core Collection, BIOSIS Citation Index, Current Contents Connect, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index, and SciELO Citation Index). No date or year restrictions were applied. MeSH terms "NLRP3", "cardiovascular disease", "atherosclerosis", "coronary artery disease," and "heart disease" were used. Data filters were applied to exclude reviews, editorial materials, books, letters, and news. This search strategy was reviewed by the second author (MK) based on the PRESS 2015 Peer Review of Electronic Strategies. Supplementary table 1 (S1) details both search strategies used.

The initial search yielded 376 articles, 293 from the Web of Science database and 83 from OVID Medline. Forty five duplicate articles were removed, 36 by the Endnote automation tool and 9 manually by two authors. Independent abstract screening by authors 1 and 2 led to removal of 294 of the remaining 331 articles. Any disagreements were resolved by author 3. From the 37 remaining articles, 17 were removed after full-text analysis based on exclusion criteria. The final number of included studies was 20. This is summarized in Fig. 1 which demonstrates a summarized PRISMA flow diagram. No other online searches, organizations, or journals were consulted.

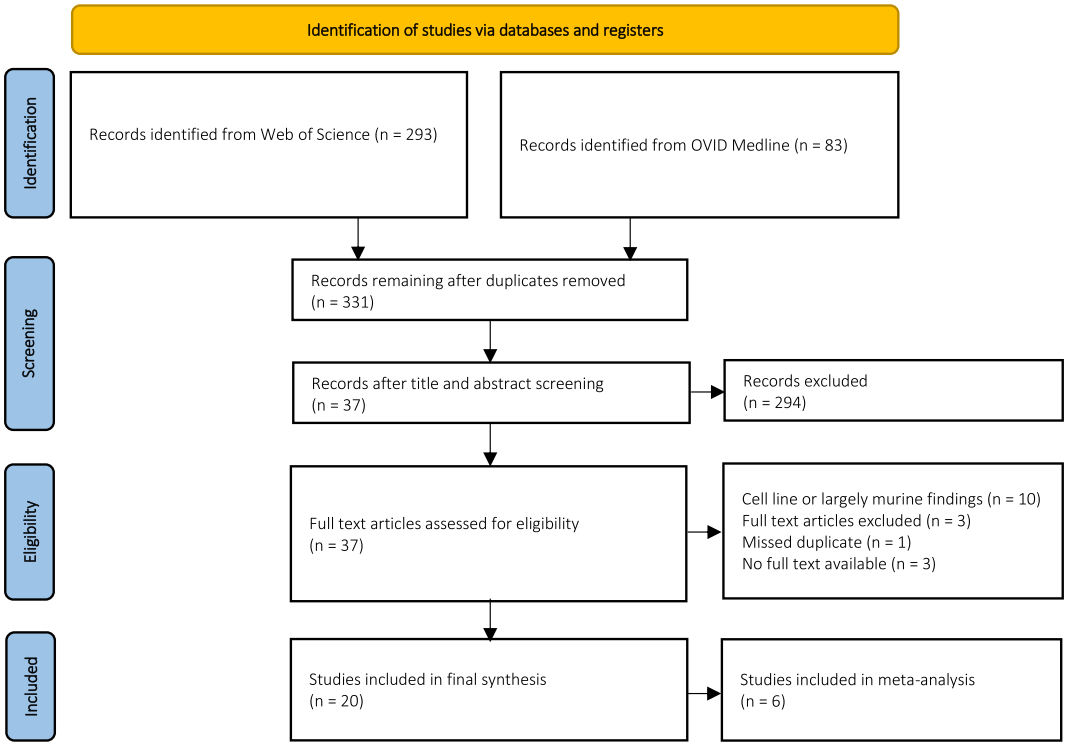

Fig. 1. PRISMA flow diagram.

Eligibility criteria

Participants over the age of 18 years with angiographic evidence of atherosclerosis or a clinical diagnosis of stable angina or acute coronary syndrome (ACS) (unstable angina or myocardial infarction) were included. Our data also included studies with primary human cell data that assessed NLRP3 and potential upstream activators such as smoking, oxidative stress, or NLRP3 drug targets. No study identified only a subset of the eligibility criteria.

Exclusion criteria

Reviews, studies irrelevant to the topic of interest, studies containing non-human or human cell line data, and of non-English language were excluded. Studies were also excluded if no full access was available or if they assessed the role of NLRP3 in diseases with etiology apart from atherosclerosis such as aortic dissection and aortic aneurysms. No other restrictions on sex, race, setting of study, or other conditions were made. The inclusion and exclusion criteria for this study were prespecified.

Data extraction

For the included studies, key information such as author, year, country of study, and number of participants were extracted into a table. The major findings of each study were summarized. Where incomplete data were reported, the study authors (12 in total) were contacted directly, two of whom provided additional data. This process was conducted independently by two authors (MK and MK) and any disputes were resolved by the third author (VV).

Data collection and statistical analysis

Expression levels of NLRP3, IL-1β, IL-18, and IL-6 and the relative fold change in mRNA expression of NLRP3, ASC, and caspase-1 were of interest in our study. Initially, data were manually extracted and tabulated, where possible, from each study pertaining to these findings. Six studies included data which were manually extracted. The remaining data were extracted from figures and graphs using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer) [20]. When possible, automation tools were used to extract data points which were then adjusted for accuracy using the manual tool in this program. Wan's method was used to convert medians to means where required [21]. Standard deviation (SD) was calculated using interquartile range (IQR) whereby $SD = \frac{IQR}{1.35}$ where required [22]. SD was calculated using standard error of measurement (SEM) where required [23]. Data and patient baseline were tabulated as mean ± SD. This was inputted into RevMan 5.4.1. (Cochrane. Revman 5.4.1. RevMan | Cochrane Training. https://training.cochrane.org/online-learning/core-software/revman. Accessed August 8, 2022). This tool allowed for: (i) calculation of mean differences, (ii) pooling of all mean differences with 95 % confidence intervals (CI) via a random-effects model, and (iii) heterogeneity exploration through chi-square and inconsistency squared. A continuous data set and inverse variance were used.

Eligibility for synthesis

Studies were included in the meta-analysis if they assessed the fold change of mRNA of NLRP3 inflammasome components or downstream cytokines. Due to different study designs, raw expression levels could not be accurately meta-analyzed. Studies that did not specifically assess CAD as a case group were excluded. Case groups were then divided into control or high-risk. Controls were consistent with stated controls in each study or when no control was present, non-smokers non-CAD, carotid endarterectomy (stable plaque) and diabetic stable CAD patients were allocated as controls. Groups were allocated as high-risk (denoted as * in figures), if they were non-smokers CAD, carotid endarterectomy (unstable plaque group), diabetic STEMI, acute myocardial infarction (AMI), or were coronary artery bypass grafts (CABG) candidates as in supplementary table 2 (S2).

Risk of bias assessment

The SIGN checklist was used by two reviewers (MK and MK) independently for assessing the risk of bias, in all twenty studies (Health Improvement Scotland SIGN. Checklists. SIGN. https://www.sign.ac.uk/what-we-do/methodology/checklists/. Accessed August 8, 2022). Only one study by Zaidi et al. was considered to have high risk of bias as it was a sub-study and reported an ad hoc analysis (refer to supplementary table 3 (S3)) [24].

Study quality assessment

For an appraisal of the quality of the included studies, the NIH: NHLBI tool was utilized. (NIH: National Heart Lung and Blood Institute. Study Quality Assessment Tools. National Heart Lung and Blood Institute. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools. Accessed August 8, 2022.) Studies were assessed by an evaluation of controls, blinding, and presence of confounding variables. Quality assessment was undertaken independently by two reviewers (MK and MK) and disputes were resolved by the third author (VV). All studies were of acceptable quality, however three were considered of poor quality (refer to supplementary table 4 (S4)) [25–27].

Results

Study characteristics

Twenty studies were included [24–42]. Table 1 provides a summary of key characteristics from included studies. Out of 20 included studies, 4 studies looked at effects of activators such as smoking, CC, and adenosine triphosphate (ATP) [28,31,40,43]. Ten studies assessed the expression of NLRP3 in human atherosclerotic disease, and effects of different polymorphisms [26,27,33,34,37,38,40–43]. Six studies evaluated the association between NLRP3 or its counterparts (ASC and caspase-1) and downstream cytokine levels including IL-1β, IL-18, and IL-6 [32,33,35,38,39,43]. Five studies investigated NLRP3 as a prognosticator of CAD severity [30,32,33,36,40]. Finally, four studies evaluated interventions such as lifestyle changes, colchicine, and rosuvastatin on NLRP3 expression [24,30,38,39]. Across all 20 selected studies, there was a total of 3388 patients. The mean sample size was 169, with a mean age of 58.92 years. Data from the studies included in the meta-analysis are summarized in supplementary table 5 (S5). Other data collected for outcomes of interest are tabulated in supplementary table 6 (S6).

1. Expression of NLRP3 in atherosclerotic disease and related polymorphisms

An emerging body of evidence has suggested a high expression level of NLRP3 in atherosclerotic disease and four included studies have supported such findings [33,34,38,43]. Multiple studies supported such findings in peripheral blood mononuclear cells (PBMCs) and carotid atherosclerotic plaques, by comparing mRNA levels to controls [33,38,43]. This is corroborated by findings of increased protein expression of NLRP3 components, ASC, and caspase-1, along with downstream cytokines IL-1β and IL-18 in atherosclerotic patients compared to controls [33,38,43]. The meta-analysis determined that NLRP3 mRNA was greater in the high-risk group compared to controls as seen in Fig. 2 [*fold change* = 0.84 (95 % CI: 0.44–1.28)] [26–28,33,36,38].

Hermansson et al. contradicts these findings, reporting a lower NLRP3 expression in ischemic cardiac tissue compared to controls in a small case study [26]. However, three of the five patients in the case group, had an

Table 1
Summary of baseline characteristics of included studies.

Number	Author	Year	Location	Sample size	Sample groups	Baseline		
						Mean age (years)	Male (%)	BMI
1	MEHTA [4]	2020	India	80	*N* = 20 non-smokers, non-CAD	46	100	24.2
					N = 20 smokers only	47	100	24.4
					N = 20 CAD only	51	100	24.8
					N = 20 CAD smokers	47.5	100	24.2
2	SAMSTAD [32]	2014	Norway	43	*N* = 22 stable angina patients	N/A	N/A	N/A
					N = 21 ACS patients	N/A	N/A	N/A
3	MARTINEZ [33]	2015	Australia	83	*N* = 10 controls	61.3	70	N/A
					N = 33 stable CAD	61.1	91	N/A
					N = 40 ACS	64.5	93	N/A
4	ROSSI [34]	2014	Italy	40	*N* = 15 controls	49	53	24.5
					N = 25 smokers	47	44	24.9
5	AFRASYAB [35]	2016	China	113	*N* = 30 controls	58.4	50	24.7
					N = 93 ACS	61.2	51.6	25.1
6	ZAIDI [30]	2019	Norway	137	*N* = 68 controls (T2DM and CAD)	63	79	28.2
					N = 69 exercise intervention (T2DM and CAD)	64	88	29
7	SHI [7]	2015	China	60	N = 10 controls for IHC	N/A	N/A	N/A
					N = 20 controls for atherosclerotic RF and ELISA	62.9	60	N/A
					N = 30 CEA carotid atherosclerotic plaques	66.5	83	N/A
					N = 15 Stable plaque	65.44	73	N/A
					N = 15 Unstable plaque	67	93	N/A
8	MAHENDRA [36]	2021	India	70	*N* = 35 chronic periodontitis only	43.42	NR	23.65
					N = 35 chronic periodontitis and CAD	56.45	NR	24.84
9	SHATERI [37]	2021	Iran	68	N = 30 controls	51.6	53	25.5
					N = 38 CAD	53.5	65.8	26.4
10	LI [3]	2020	China	80	*N* = 13 non-DM stable CAD	61	61.5	26.2
					N = 36 non-DM STEMI	59.5	60	24.8
					N = 10 DM stable CAD	61	66.7	25.3
					N = 21 DM STEMI	67	71.4	27.4
11	CLEMENT [1]	2019	Not reported	18	N = 9 adjacent control areas	71.2	99	N/A
					N = 9 carotid endarterectomy patient samples	71.2	100	N/A
12	ZHAO [38]	2016	China	576	*N* = 281 controls/stenosis <50 %	54.34	46.98	24.77
					N = 295 cases/stenosis >50 %	58.62	72.2	25.1
13	ZHU [9]	2019	China	60	N = 20 controls	59	6	24.99
					N = 20 SAP	64.05	11	25.57
					N = 20 AMI	64.05	12	25.73
14	AFRASYAB [39]	2015	China	123	N = 30 controls	58.43	15	24.67
					N = 40 UA	61.35	20	25.08
					N = 53 AMI	61.19	28	25.17
15	ZHENG [40]	2013	China	46	N = 36 CABG candidates	NR	NR	NR
					N = 10 controls	NR	NR	NR
16	AKOSILE [41]	2020	Australia	299	*N* = 299 Vietnamese veterans	NR	100	NR
					N = 0 controls	NR	NR	NR
17	ZHOU [42]	2016	China	916	*N* = 401 controls	57.49	60.1	24.18
					N = 515 CAD	59.5	70.68	24.75
18	RAJAMAKI [43]	2016	Finland	10	N = 6 cardiac transplant patients	57.6	100	NR
					N = 4 cardiac transplant donor controls	57.16	100	NR
19	HERMANSSON [2]	2013	Sweden	8	N = 5 CABG candidates	NR	NR	NR
					N = 3 controls	NR	NR	NR
20	CHENG [31]	2018	China	558	*N* = 293 LAA stroke patients	62.89	74.7	23.98
					N = 265 controls	61.27	56.6	23.87

Abbreviations: CAD (coronary artery disease); ACS (acute coronary syndrome); T2DM (type 2 diabetes mellitus); IHC (immunohistochemistry); ELISA (enzyme-linked immunosorbent assay); CEA (carotid endarterectomy); DM (diabetes mellitus); SAP (stable angina pectoris); AMI (acute myocardial infarction); UA (unstable angina); LAA (large artery atherosclerosis).

unknown expression pattern or a polymorphism in the Q705K region of the NLRP3 gene [26]. These mutations were absent in the nonischemic control group [26]. Studies investigating NLRP3 single nucleotide polymorphisms (SNPs) found that certain SNPs (RS10159239, RS10754558, RS4612666) are more strongly associated with CAD; which could explain the findings by Hermansson et al. [26,37,40–42].

2. Inducers of NLRP3 expression and activation

Our review focuses on CC, ATP, smoking and, oxidative stress as NLRP3 activators.

A. CC and lipid profiles

Three human studies examined the role of CCs in NLRP3 inflammasome activation [29,40,43]. In a study conducted by Rajamaki et al., human monocyte derived macrophages (HMDMs) were treated with CCs with a notable downregulation of NLRP3 expression. Importantly, this effect was reversed when HMDMs were primed with LPS, a known inducer of NLRP3 expression, and subsequently treated with CC [43].

The second study by Zheng et al. demonstrated a positive association between NLRP3 expression, total cholesterol, low-density lipoprotein (LDL), and lipoprotein a [Lp(a)] [40]. Importantly, for the first time in a human study, NLRP3 inflammasome expression was inversely associated with high-density lipoprotein cholesterol (HDL—C) [40].

Samstad et al. hypothesized that CC induced the production of reactive oxygen species (ROS) acting as a DAMP leading to the activation of NLRP3 [29]. Rajamaki's study also explored detailed mechanisms involving p38-δ mitogen-activated protein kinase (MAPK) and ATP in CC-induced NLRP3 inflammasome activation, as summarized in Fig. 3 [43].

i. Complement system activation

Samstad et al. reported that CC induced the classic (terminal complement complex and C3bc) and alternative (C3b-Bb-properdin

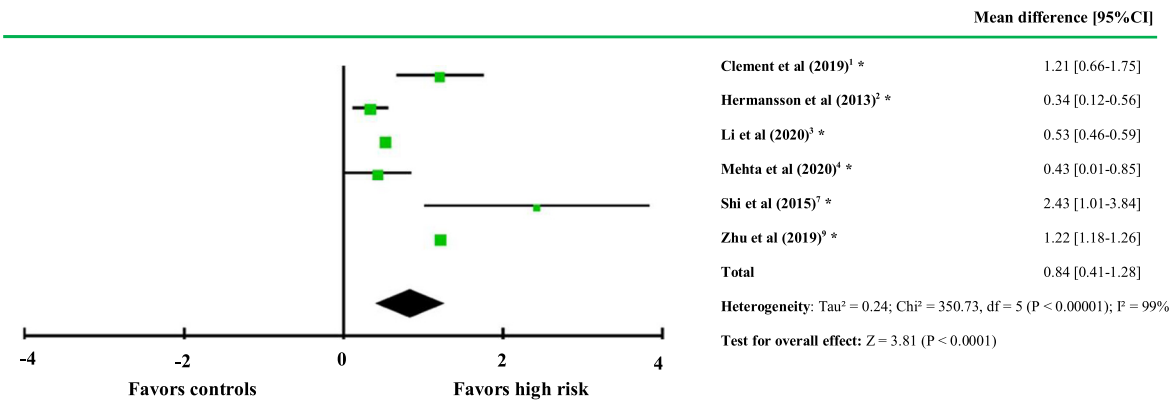

Fig. 2. Fold change of NLRP3 mRNA levels in controls vs. high-risk atherosclerotic disease.
Note*: Groups were allocated as high-risk (denoted as * in figures) if they were non-smokers CAD, CEA (unstable plaque group), diabetic STEMI, AMI, or were CABG candidates (see eligibility for synthesis for further information).

complex, C3BbP) complement pathway in a time-dependent manner [29]. They also noted the importance of C5 and its cleavage to C5a in CC-induced phagocytosis, ROS production, IL-1β production as all three parameters were attenuated by C5-cleavage and C5aR inhibitors. Importantly, these effects were restored by C5 replacement [29].

ii. p38-δ MAPK and ATP

It was noted that p38 MAPKs and caspase-1 activation increased when both un-primed and LPS-primed HMDMs were treated with CCs. Additionally, whilst IL-1β levels increased in both groups, the rise was more substantial in LPS-primed HMDMs [43]. Furthermore, ATP independently induced the activation of p38-δ MAPK via interaction with the P2X7 receptor [31,43].

To investigate the role of different p38 MAPK isoforms, two non-selective inhibitors, SB203580 (p38-α/β inhibitor) and BIRB-796 (p38α/β/γ/δ inhibitor) were used [43]. BIRB-794 had a dose-dependent inhibition of pro-IL-1β and subsequently, mature IL-1β in LPS-primed HMDMs. It also attenuated the activation of p38-δ MAPK by ATP [31,43]. Interestingly, these effects were not observed with SB203580 [43].

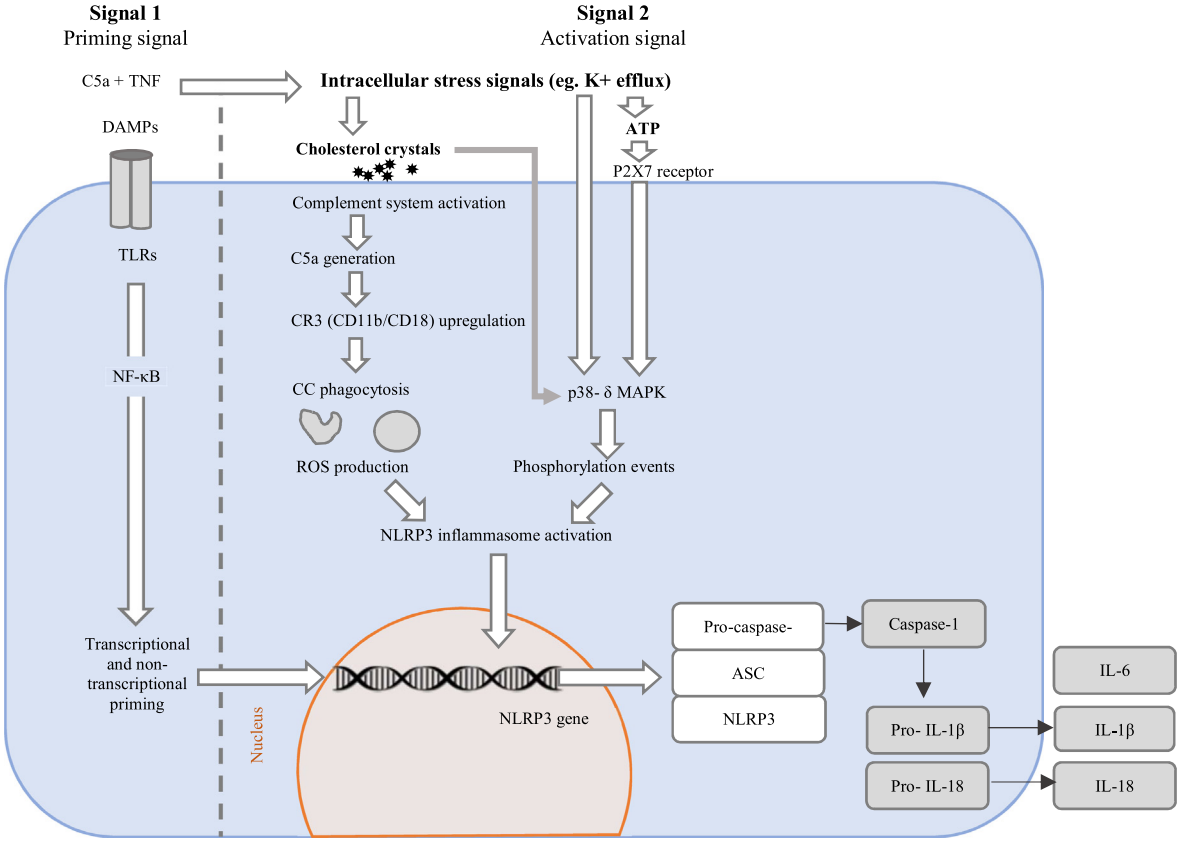

Fig. 3. Canonical activation of NLRP3 inflammasome activation. This diagram summarises the two-step canonical activation of the NLRP3 inflammasome consisting of a priming signal and an activation signal. C5a (complement 5a); TNF (tumor necrosis factor); DAMPs (damage-associated molecular patter; TLR (toll-like receptor); NF-kB (nuclear factor kappa light-chain-enhancer of activated B cells); CR3 (complement-receptor 3); CC (cholesterol crystals); ROS (reactive oxygen species); ATP (adenosine triphosphate); P2X7 (purinoreceptor 7); p38- δ MAPK (mitogen-activated protein kinase); NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3); ASC (apoptosis-associated speck-like protein); IL-1β, 6 and 18 (interleukin).

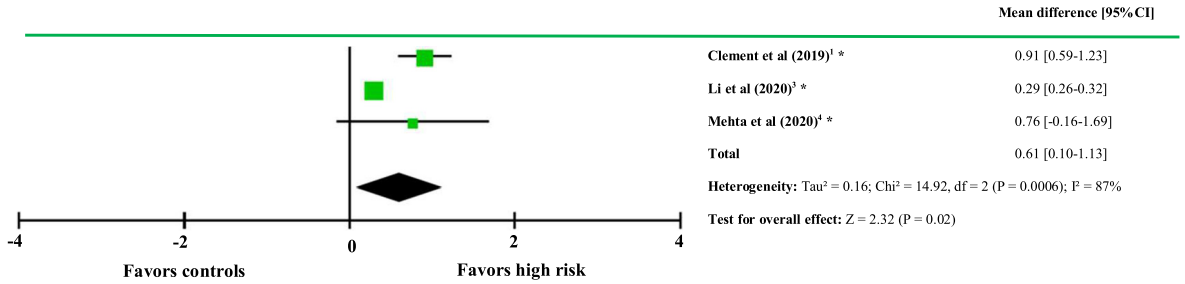

Fig. 4. Fold change of IL-1β mRNA levels in controls vs. high-risk atherosclerotic disease.
Note*: Groups were allocated as high-risk (denoted as * in figures) if they were non-smokers CAD, CEA (unstable plaque group), diabetic STEMI, AMI, or were CABG candidates (see eligibility for synthesis for further information).

B. Smoking

Two studies evaluated smoking and NLRP3 inflammasome activation [28,31]. Rossi et al. reported that NLRP3 and downstream cytokines were more expressed in smokers compared to non-smokers [31]. Mehta et al. corroborated this finding and reported a dose-dependent effect of smoking on NLRP3 expression in CAD [28]. Additionally, a positive correlation was noted between serum cotinine, a known metabolite of nicotine, and NLRP3 markers [28]. It was stated that smoking induced a milieu of oxidative stressors, which activated NLRP3, contributing to atherosclerosis progression and complications [28].

Rossi et al. hypothesized that the P2X7 receptor mediates smoking-related atherosclerosis. This hypothesis was initially due to linear correlations between P2X7 and NLRP3, IL-1β, and IL-18. Further supporting evidence showed a reduction in IL-1β and IL-18 when a human P2X7 antagonist was used [31].

3. Downstream cytokine production from NLRP3

Multiple studies linked NLRP3 activation with production of downstream cytokines [35,39]. Shateri et al. noted that NLRP3 and IL-1β mRNA were concurrently increased in CAD compared to controls. Furthermore, it was noted that NLRP3 activation also had a positive correlation with IL-18 expression [35]. Finally, the meta-analysis demonstrated a greater expression of NLRP3, IL-1β, and IL-18 in high-risk compared to control groups (Figs. 2, 4, 5).

4. Prognostication

A. NLRP3 is associated with CAD severity and poorer outcomes

Novel research established a link between NLRP3 expression and severity of CAD in 2 out of 20 studies [32,33]. Two studies also examined NLRP3 as a prognosticator in CAD severity tools [32,40].

Afrasyab et al. demonstrated a positive correlation between NLRP3 protein levels and CAD severity, measured by degrees of vessel disease, both angiographically and grossly [32]. This is supported by higher NLRP3 mRNA expression in unstable atherosclerotic plaques compared to stable plaques in CEA patients [33]. NLRP3 protein levels were correlated to various angiographic scoring systems including, Gensini, SYNTAX, and the Clinical SYNTAX score (cSS) [32,40].

Measures and scoring systems predicting poorer outcomes including GRACE ACS, thrombolysis in myocardial infarction (TIMI), and major cardiac adverse events (MACE) were positively correlated with NLRP3 expression [32]. Additionally, Li et al. noted higher levels of NLRP3 in STEMI patients compared to stable coronary heart disease patients [36].

B. Cytokines correlated with CAD severity

Three out of twenty studies further linked NLRP3-related cytokines with CAD severity [30,33,36]. In a study by Martinez et al., transcoronary gradients of IL-1β, IL-18, and IL-6 were present in higher levels in ACS subjects, compared to stable CAD and controls [30]. Similar findings were noted for IL-1β and IL-18 in two other studies [33,36].

5. Role of interventions

Four included studies explored three interventions – exercise, rosuvastatin, and colchicine – affecting NLRP3 [24,30,38,39].

A. Exercise

Zaidi et al. investigated the effect of 12-month exercise training in patients with type 2 diabetes mellitus and CAD on the circulating levels and gene expression of NLRP3, associated cytokines in leucocytes, and

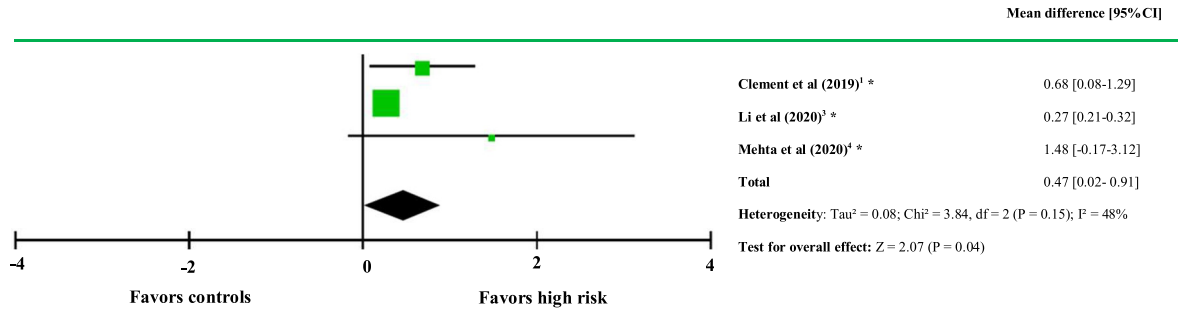

Fig. 5. Fold change of IL-18 mRNA levels in controls vs. high-risk atherosclerotic disease.
Note*: Groups were allocated as high-risk (denoted as * in figures) if they were non-smokers CAD, CEA (unstable plaque group), diabetic STEMI, AMI, or were CABG candidates (see eligibility for synthesis for further information).

subcutaneous adipose tissue [24]. No statistically significant difference was found.

B. Statins

In two studies, NLRP3, IL-1β, and IL-18 expression was reduced by rosuvastatin [38,39]. Additionally, a dose-dependent reduction in NLRP3 and downstream cytokines was observed when comparing a 5 mg/day to a 20 mg/day regimen [39].

C. Colchicine

Martinez et al. reported a significant decrease in trans-coronary levels of IL-1β and IL-18 in ACS patients when treated acutely with colchicine [30]. This was suggested to result from the inhibitory effects of colchicine on NLRP3 [30].

Discussion

The meta-analysis suggests that NLRP3, and downstream cytokines, IL-1β and IL-18 are more greatly expressed in high-risk atherosclerotic disease compared to controls. This finding supports the implication of NLRP3 inflammasome activation and downstream release of pro-inflammatory cytokines in the pathogenesis and progression of atherosclerotic disease.

Several activators of NLRP3 inflammasome activation have been studied. CCs, specifically those comprising LDL and Lp(a), were found to induce the activation of NLRP3 by acting as DAMPs; whilst HDL-C was negatively associated with NLRP3 expression [29,40]. Through the investigation of p38 MAPK isoform inhibitors, it can be hypothesized that the downstream effects of NLRP3 due to CC is mediated by ATP and the δ and γ isoforms of p38 [31,43]. Smoking was noted to have a dose-dependent relationship with NLRP3 expression, by inducing ROS and known oxidative stress markers [28,44]. Similarly, through the use of P2X7 inhibitors in peripheral vascular adipose tissue (PVAT), it was noted that the effect of smoking on NLRP3 activation, and downstream cytokine production is likely mediated through the ATP receptor, P2X7 [31]. Whilst PVAT may play a role in the pathogenic microenvironment of atherosclerosis, its relevance is not completely understood [45].

Despite the large emerging body of evidence in our review that supports a role for NLRP3 in atherosclerosis, one study raised contradictory findings. Hermansson et al., noted that human ischemic cardiac tissue had a reduced expression of NLRP3 compared to controls [26]. However, two of the patients in the small case group of five had NLRP3 polymorphisms in the Q705K region, which were absent in the non-ischemic control group [26]. Additionally, these polymorphisms present in the case group, were associated with more severe inflammation, and higher levels of IL-1β and IL-18 [26]. These mutations, in conjunction with the small sample size, more adequately explain the findings by Hermansson et al., rather than a disassociation between atherosclerosis and NLRP3.

Given the potential association between the NLRP3 inflammasome, downstream cytokines and atherosclerosis, therapies targeting these may be favorable in reducing atherosclerosis-related complications. The CANTOS study demonstrated that IL-1β inhibition with canakinumab resulted in a reduction in cardiovascular events and high-sensitivity C-reactive protein levels, implicating the causal role of inflammation in atherosclerosis [19]. Further, the Low-Dose Colchicine (LoDoCo2) trial showed a 31 % reduction in cardiovascular mortality, ischemic stroke, and myocardial infarction after one month of low-dose colchicine in stable chronic CAD patients compared to patients receiving placebo [46,47]. The Colchicine Cardiovascular Outcomes Trial (COLCOT) trial also demonstrated markedly reduced IL-1β and caspase-1 production in ACS patients on short-term colchicine [48]. Similarly from our review, Martinez's study showed that colchicine administration in unstable CAD patients reduced local synthesis of IL-1β, IL-18, and IL-6 [30]. Collectively, these findings support the promising advantages of anti-inflammatory agents targeting NLRP3 and its downstream cytokines in CAD patients [19,30,46–49].

Our study has several strengths. We performed a systematic review of the literature that expanded the number of studies compared to previous reviews. The review is also unique in its focus on primary human data only. Furthermore, one of the strengths of this meta-analysis is the use of a thorough search strategy across various databases to ensure all suitable studies were included. Our review and meta-analysis also provided pooled mean differences using the random-effects model addressing heterogeneity.

In relation to the meta-analysis, included studies had a large variation in sample sizes, blinding, and inconsistent definitions for cases and controls. Such variability contributed to significant clinical and methodological heterogeneity. Additionally, the variation in methodology and data collection, as highlighted in the risk of bias assessment, led to difficulty with result comparability, reducing the effective sample size and meta-analysis power. As this was the first to use a meta-analysis evaluating NLRP3, IL-1β, and IL-18 in atherosclerotic disease, we could not compare our findings. Furthermore, in some studies, raw data had to be extracted from graphs, which is a limitation to the accuracy of our findings. Despite such limitations, this systematic review and meta-analysis met the initial objectives and was the first to explore NLRP3 in human studies in the context of atherosclerosis.

Conclusion

This systematic review evaluated the role of the NLRP3 inflammasome in atherosclerosis. From the analysis, NLRP3 as well as its known pro-inflammatory cytokines, IL-1β and IL-18, was associated with high-risk atherosclerotic disease. It is our view that several activators, particularly the well-established role of CC and smoking, are involved in the activation of the NLRP3 inflammasome and its downstream cytokines. From this review, evidence for drug targets such as rosuvastatin and colchicine in targeting NLRP3 and its cytokines is possible, but evidence is limited to small sample clinical studies. Nonetheless, it is recommended that future, multi-center human studies are conducted to evaluate the role of therapeutics and further delineate their potential role in managing atherosclerosis through the mediation of NLRP3.

Ethics statement

All analyses included in this systematic review were based on previously published studies; no ethical approval or patient consent was required.

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CRediT authorship contribution statement

Marina Khair: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Mark Khair:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Venkat N. Vangaveti:** Supervision, Methodology, Formal analysis, Writing – review & editing. **Usman H. Malabu:** Supervision, Writing – review & editing.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jjcc.2024.03.003.

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