

# Classical Pathway of Complement Activation: Longitudinal Associations of C1q and C1-INH With Cardiovascular Outcomes: The CODAM Study (Cohort on Diabetes and Atherosclerosis Maastricht) Brief Report

Citation for published version (APA):

Hertle, E., Arts, I. C. W., van der Kallen, C. J. H., Feskens, E. J. M., Schalkwijk, C. G., Stehouwer, C. D. A., & van Greevenbroek, M. M. J. (2018). Classical Pathway of Complement Activation: Longitudinal Associations of C1q and C1-INH With Cardiovascular Outcomes: The CODAM Study (Cohort on Diabetes and Atherosclerosis Maastricht) Brief Report. *Arteriosclerosis Thrombosis and Vascular Biology*, 38(5), 1242-1244. <https://doi.org/10.1161/ATVBAHA.118.310806>

## Document status and date:

Published: 01/05/2018

## DOI:

[10.1161/ATVBAHA.118.310806](https://doi.org/10.1161/ATVBAHA.118.310806)

## Document Version:

Publisher's PDF, also known as Version of record

## Document license:

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# Classical Pathway of Complement Activation: Longitudinal Associations of C1q and C1-INH With Cardiovascular Outcomes

## The CODAM Study (Cohort on Diabetes and Atherosclerosis Maastricht)—Brief Report

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**Objective**—The classical complement pathway has been assigned both protective and pathological effects in cardiovascular disease (CVD), but human data are lacking. We determined the associations of the pattern recognition factor C1q and the regulator C1-INH (C1-inhibitor) with incident CVD, carotid intima–media thickness, endothelial dysfunction, and low-grade inflammation.

**Approach and Results**—Baseline concentrations of C1q and C1-INH were measured in the CODAM study (Cohort on Diabetes and Atherosclerosis Maastricht; n=574; 61% men; age, 60±7 years). The 7-year incidence of CVD in participants free of CVD at baseline was evaluated using logistic regression analyses (n=342; 73 cases). The lowest incidence of CVD was observed in the middle tertile of C1q ( $T_{\text{low}}$  compared with  $T_{\text{middle}}$ : odds ratio, 2.38 [95% confidence interval, 1.14–4.95];  $T_{\text{high}}$  compared with  $T_{\text{middle}}$ : odds ratio, 1.96 [95% confidence interval, 0.94–4.07]). C1-INH was not associated with CVD. During the 7-year follow-up period, C1q and C1-INH were not, or inconsistently, associated with carotid intima–media thickness or with biomarker scores reflecting endothelial dysfunction and low-grade inflammation.

**Conclusions**—Our results suggest a nonlinear association between C1q and incident CVD. This supports the concept that early steps in classical pathway activation may have both protective and pathological effects on human CVD.

**Visual Overview**—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2018;38:1242-1244. DOI: 10.1161/ATVBAHA.118.310806.)

**Key Words:** cardiovascular diseases ■ carotid intima–media thickness ■ follow-up studies ■ humans ■ incidence

The classical pathway of the complement system is initiated by recognition of danger signals, such as apoptotic cells, immune complexes, CRP (C-reactive protein)-marked surfaces, and modified lipoproteins.<sup>1,2</sup> Experimental studies showed that pattern recognition-induced complement activation can lead to silent removal of unwanted products without full complement activation,<sup>3</sup> which is considered beneficial. On the other hand, extensive classical pathway activation and progression to terminal complement activation can induce pro-inflammatory signaling,<sup>2,4</sup> which may contribute to cardiovascular pathology. Therefore, the classical pathway is thought to have both protective and pathological effects in cardiovascular disease (CVD).<sup>2</sup> Currently, no longitudinal human data on the classical pathway in CVD are available. Therefore, we measured in a prospective human cohort (CODAM study [Cohort on Diabetes and Atherosclerosis Maastricht]) C1q, the recognition molecule and activator of the classical pathway, and C1-INH (C1-inhibitor), the main factor that controls classical

pathway activation. During a 7-year follow-up period, we investigated the associations of baseline C1q and C1-INH with incident CVD and cardiovascular events (CVEs), with biomarker scores reflecting low-grade inflammation and endothelial dysfunction and with carotid intima–media thickness (cIMT) as a reflection of atherosclerosis.

### Materials and Methods

CODAM is a prospective cohort study with a 7-year follow-up.<sup>5</sup> All participants were extensively phenotyped, as described before.<sup>5,6</sup> Complement C1q and C1-INH were measured in EDTA plasma by ELISA (C1q as described in the study by Dillon et al,<sup>7</sup> and C1-INH: MicroVue C1-INH EIA kit; Quidel, San Diego). Generalized estimating equations and multiple logistic regression were used to evaluate the associations of baseline C1q or C1-INH with inflammation, endothelial dysfunction and cIMT, and with incident CVD and CVE, respectively. Several sensitivity analyses were done to evaluate the robustness of the findings. Extensive description of methods is available in Materials in the [online-only Data Supplement](#).

Received on: July 14, 2017; final version accepted on: February 27, 2018.

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The online-only Data Supplement is available with this article at <http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.118.310806/-/DC1>.

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*Arterioscler Thromb Vasc Biol* is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.118.310806

### Nonstandard Abbreviations and Acronyms

<b>cIMT</b>	carotid intima–media thickness
<b>CODAM</b>	Cohort on Diabetes and Atherosclerosis Maastricht
<b>CRP</b>	C-reactive protein
<b>CVD</b>	cardiovascular disease
<b>CVE</b>	cardiovascular event
<b>IL</b>	interleukin
<b>sICAM-1</b>	soluble intercellular adhesion molecule 1
<b>sVCAM-1</b>	soluble vascular adhesion molecule 1
<b>TNF</b>	tumor necrosis factor

## Results

Baseline characteristics of the study population as a whole ( $n=547$ ; Table I in the [online-only Data Supplement](#)) and across tertiles of C1q (Table IIA in the [online-only Data Supplement](#)) and C1-INH (Table IIB in the [online-only Data Supplement](#)) are provided. Because experimental studies suggested both protective and pathological effects in CVD, nonlinear associations with cardiovascular outcomes could be anticipated. Therefore, deviation from linearity was checked in all analyses. In logistic regression analyses on incident CVD ( $n=73$ ) and CVE ( $n=39$ ) in individuals free of CVD at baseline ( $n=342$ ), we observed clear deviations from linearity for associations of C1q with incident CVD and CVE. Participants in the middle tertile of C1q had the lowest incidence of CVD; therefore,  $T_{\text{middle}}$  was chosen as reference (Figure). In the fully adjusted model, the odds ratio for CVD of  $T_{\text{low}}$  compared with  $T_{\text{middle}}$  of C1q was 2.38 (95% confidence interval, 1.14–4.95), and the odds ratio of  $T_{\text{high}}$  compared with  $T_{\text{middle}}$  was 1.96 (95% confidence interval, 0.94–4.07). This suggested a potentially nonlinear relation between C1q and incident CVD. For incident CVE, a similar pattern was observed (Figure), but the observed odds ratios did not reach statistical significance. C1-INH was not associated with incident CVD or CVE (Figure I in the [online-only Data Supplement](#)).

In the generalized estimating equation analyses of baseline C1q or C1-INH with the inflammation score (ie, the average of the standardized values of CRP, IL [interleukin]-6, IL-8, TNF [tumor necrosis factor]- $\alpha$ , sICAM-1 [soluble intercellular adhesion molecule-1], and serum amyloid A), the endothelial dysfunction score (ie, the average of the standardized values of von Willebrand factor, sE-selectin, sICAM-1, and sVCAM-1 [soluble vascular adhesion

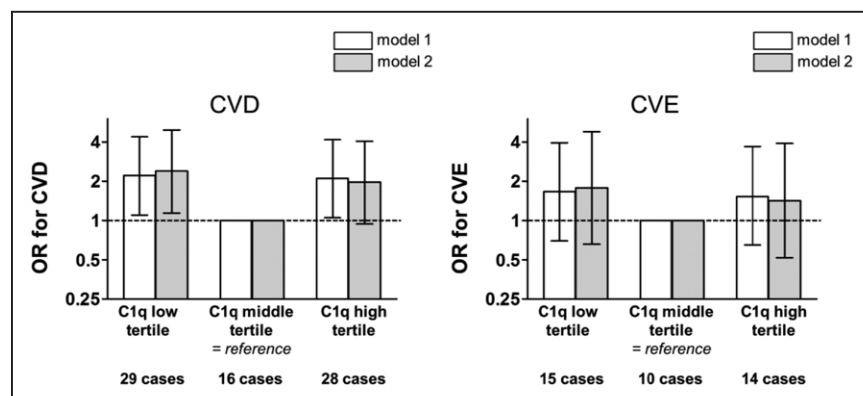
molecule 1]) or cIMT, we did not observe deviations from linearity, and C1q and C1-INH were subsequently analyzed as continuous variables. To evaluate whether the strength of these associations was stable over time, an interaction term between time and C1q or C1-INH was included in all generalized estimating equation models. In the associations between C1q and the inflammation score and the endothelial dysfunction score, this interaction term was significant. Therefore, baseline and follow-up observations were analyzed separately. C1q was positively associated with the inflammation score and the endothelial dysfunction score at baseline (Table III in the [online-only Data Supplement](#); per 1 SD higher C1q,  $\beta$  for the inflammation score was 0.09 [95% confidence interval, 0.01–0.16];  $\beta$  for the endothelial dysfunction score was 0.14 [95% confidence interval, 0.07–0.21]) but not at follow-up (all  $P$  values  $>0.05$ ), indicating lack of a prospective association. C1q was not associated with cIMT ( $P>0.05$ ). C1-INH was not associated with the inflammation score, the endothelial dysfunction score, or cIMT (Table III in the [online-only Data Supplement](#); all  $P$  values  $>0.05$ ; no interaction with time).

These observations were similar when individuals with diabetes mellitus (Figure II in the [online-only Data Supplement](#)) or with a potential acute inflammation (CRP,  $>10$  mg/L) were excluded. All associations were comparable in men and women ( $P$  value for interaction,  $>0.05$ ).

## Discussion

The main finding of this study is that both high and low plasma concentrations of C1q were associated with a 2- to 2.5-fold higher risk to develop CVD, with a similar trend for CVE. This implies that C1q may have both protective and pathological effects in CVD. Beneficial effects of C1q on immune regulation<sup>8</sup> combined with adverse effects on metabolism<sup>9</sup> could underlie the observed relation between C1q and CVD.

At first sight, our findings may contrast with previous studies that did not report an association between C1q and prevalent CVD.<sup>10,11</sup> However, those studies were cross-sectional, considerably smaller, and did not explore a nonlinear relationship. Our findings that low C1q concentrations are associated with higher CVD risk are consistent with the fact that genetic deficiency of C1q is associated with autoimmune diseases<sup>12</sup> that, in turn, are associated with higher CVD risk.<sup>13,14</sup> No association was observed for C1q with atherosclerosis as reflected by cIMT, in line with the only previous, smaller human study



**Figure.** Association of baseline C1q (in tertiles) with incident cardiovascular disease (CVD) and cardiovascular event (CVE). Tertiles of C1q were analyzed in 342 participants free of CVD at baseline. C1q was  $56.1 \pm 5.6$  mg/L in  $T_{\text{low}}$  ( $n=114$ ),  $71.0 \pm 3.8$  mg/L in  $T_{\text{middle}}$  ( $n=114$ ), and  $89.3 \pm 9.1$  mg/L in  $T_{\text{high}}$  ( $n=114$ ). Model 1 is adjusted for age, sex, and glucose metabolism status. Model 2 was additionally adjusted for anthropometric measures, classic cardiovascular risk factors, and medication use. OR indicates odds ratio.

on C1q and cIMT.<sup>15</sup> Because cIMT reflects thickening of the arterial wall, a role for C1q in other aspects of atherosclerosis, such as in plaque burden or plaque phenotype, is not excluded by this observation.

Notably, nonlinear associations were not observed between C1q and inflammation, endothelial dysfunction, or cIMT. C1q at baseline was positively associated with baseline markers of inflammation and endothelial dysfunction but not at follow-up. Because C1q did not predict future inflammation and endothelial dysfunction, plasma C1q may, at least partly, result from ongoing inflammatory processes rather than vice versa.

C1-INH was not independently associated with any cardiovascular outcome. C1-INH is known for its potent anti-inflammatory effects on acute inflammation, for example, in hereditary angioedema.<sup>16</sup> The lack of an association with low-grade inflammation suggests that C1-INH may be of minor relevance in obesity-associated chronic low-grade inflammation.

In conclusion, our findings support the concept that the proximal classical pathway may exert both protective and pathological effects in development of CVD as reflected by a nonlinear relation of C1q with risk of CVD.

### Sources of Funding

Part of this work was supported by grants from the Netherlands Organization for Scientific Research (940-35-034) and the Dutch Diabetes Research Foundation (98.901). The PhD fellowship of E. Hertle was supported by the Dutch Heart Foundation (NHS2010B194).

### Disclosures

None.

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### Highlights

- First prospective human study on proximal components of the classical complement pathway and cardiovascular disease.
- For C1q, classical pathway activator, and recognition molecule, a nonlinear association with incident cardiovascular disease was observed.
- The regulator of classical pathway activation, C1-INH (C1-inhibitor), was not associated with cardiovascular outcomes.