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

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

Elisabeth Hertle, Ilja C.W. Arts, Carla J.H. van der Kallen, Edith J.M. Feskens, Casper G. Schalkwijk, Coen D.A. Stehouwer, Marleen M.J. van Greevenbroek

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_{low} compared with T_{middle}: odds ratio, 2.38 [95% confidence interval, 1.14–4.95]; T_{high} compared with T_{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.[1,2] Experimental studies showed that pattern recognition-induced complement activation can lead to silent removal of unwanted products without full complement activation,[3] which is considered beneficial. On the other hand, extensive classical pathway activation and progression to terminal complement activation can induce proinflammatory signaling,[2,4] which may contribute to cardiovascular pathology. Therefore, the classical pathway is thought to have both protective and pathological effects in cardiovascular disease (CVD).[2] 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.[5] All participants were extensively phenotyped, as described before.[5,6] Complement C1q and C1-INH were measured in EDTA plasma by ELISA (C1q as described in the study by Dillon et al,[7] 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.

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From the CARIM School for Cardiovascular Diseases (E.H., I.C.W.A., C.J.H.v.d.K., C.G.S., C.D.A.S., M.M.J.v.G.), Department of Internal Medicine (E.H., C.J.H.v.d.K., C.G.S., C.D.A.S., M.M.J.v.G.), Department of Epidemiology (I.C.W.A.), and Maastricht Centre for Systems Biology (I.C.W.A.), Maastricht University, The Netherlands; and Division of Human Nutrition, Section of Nutrition and Epidemiology, Wageningen University, The Netherlands (E.J.M.F.).

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Correspondence to Marleen M.J. van Greevenbroek, PhD, Department of Internal Medicine, CARIM School for Cardiovascular Diseases, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands. E-mail m.vangreevenbroek@maastrichtuniversity.nl

Nonstandard Abbreviations and Acronyms	
cIMT	carotid intima–media thickness
CODAM	Cohort on Diabetes and Atherosclerosis Maastricht
CRP	C-reactive protein
CVD	cardiovascular disease
CVE	cardiovascular event
IL	interleukin
sICAM-1	soluble intercellular adhesion molecule 1
sVCAM-1	soluble vascular adhesion molecule 1
TNF	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_{middle} was chosen as reference (Figure). In the fully adjusted model, the odds ratio for CVD of T_{low} compared with T_{middle} of C1q was 2.38 (95% confidence interval, 1.14–4.95), and the odds ratio of T_{high} compared with T_{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]-α, 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, β for the inflammation score was 0.09 [95% confidence interval, 0.01–0.16]; β 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[8] combined with adverse effects on metabolism[9] 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.[10,11] 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[12] that, in turn, are associated with higher CVD risk.[13,14] No association was observed for C1q with atherosclerosis as reflected by cIMT, in line with the only previous, smaller human study

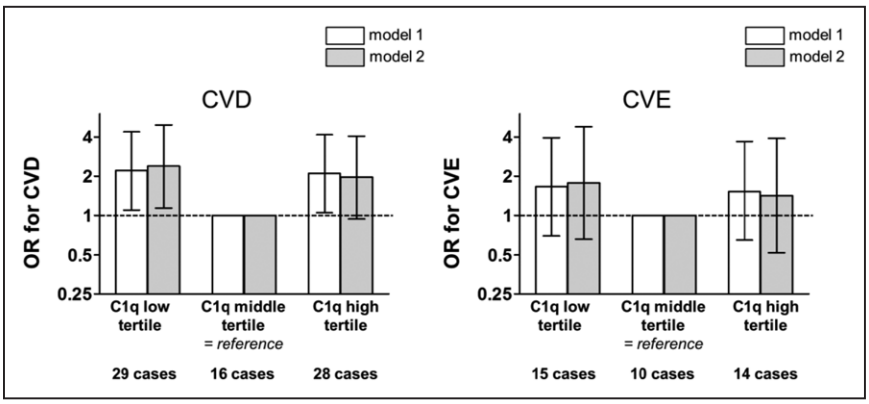

Figure. Association of baseline C1q (in tertiles) with incident cardiovascular disease (CVD) and cardiovascular event (CVE). Tertiles of C1q were analyzed in 342 participants free of CVD at baseline. C1q was 56.1±5.6 mg/L in T_{low} (n=114), 71.0±3.8 mg/L in T_{middle} (n=114), and 89.3±9.1 mg/L in T_{high} (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.[15] 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.[16] 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.

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Disclosures

None.

References

1. Trouw LA, Blom AM, Gasque P. Role of complement and complement regulators in the removal of apoptotic cells. *Mol Immunol*. 2008;45:1199–1207. doi: 10.1016/j.molimm.2007.09.008.
2. Speidl WS, Kastl SP, Huber K, Wojta J. Complement in atherosclerosis: friend or foe? *J Thromb Haemost*. 2011;9:428–440. doi: 10.1111/j.1538-7836.2010.04172.x.
3. Bohlson SS, Fraser DA, Tenner AJ. Complement proteins C1q and MBL are pattern recognition molecules that signal immediate and long-term protective immune functions. *Mol Immunol*. 2007;44:33–43. doi: 10.1016/j.molimm.2006.06.021.
4. Beinrohr L, Dobó J, Závodszky P, Gál P. C1, MBL-MASPs and C1-inhibitor: novel approaches for targeting complement-mediated inflammation. *Trends Mol Med*. 2008;14:511–521. doi: 10.1016/j.molmed.2008.09.009.
5. Wlazlo N, van Greevenbroek MM, Ferreira I, Feskens EJ, van der Kallen CJ, Schalkwijk CG, Bravenboer B, Stehouwer CD. Complement factor 3 is associated with insulin resistance and with incident type 2 diabetes over a 7-year follow-up period: the CODAM study. *Diabetes Care*. 2014;37:1900–1909. doi: 10.2337/dc13-2804.
6. Hertle E, Arts IC, van der Kallen CJ, Feskens EJ, Schalkwijk CG, Hoffmann-Petersen IT, Thiel S, Stehouwer CD, van Greevenbroek MM. Distinct longitudinal associations of MBL, MASP-1, MASP-2, MASP-3, and MAp44 with endothelial dysfunction and intima-media thickness: the cohort on diabetes and atherosclerosis maastricht (CODAM) study. *Arterioscler Thromb Vasc Biol*. 2016;36:1278–1285. doi: 10.1161/ATVBAHA.115.306552.
7. Dillon SP, D'Souza A, Kurien BT, Scofield RH. Systemic lupus erythematosus and C1q: a quantitative ELISA for determining C1q levels in serum. *Biotechnol J*. 2009;4:1210–1214. doi: 10.1002/biot.200800273.
8. Spivia W, Magno PS, Le P, Fraser DA. Complement protein C1q promotes macrophage anti-inflammatory M2-like polarization during the clearance of atherogenic lipoproteins. *Inflamm Res*. 2014;63:885–893. doi: 10.1007/s00011-014-0762-0.
9. Hillian AD, McMullen MR, Sebastian BM, Roychowdhury S, Rowchowdhury S, Kashyap SR, Schauer PR, Kirwan JP, Feldstein AE, Nagy LE. Mice lacking C1q are protected from high fat diet-induced hepatic insulin resistance and impaired glucose homeostasis. *J Biol Chem*. 2013;288:22565–22575. doi: 10.1074/jbc.M113.465674.
10. Hirata A, Kishida K, Nakatsuji H, Kobayashi H, Funahashi T, Shimomura I. High serum C1q-adiponectin/total adiponectin ratio correlates with coronary artery disease in Japanese type 2 diabetics. *Metabolism*. 2013;62:578–585. doi: 10.1016/j.metabol.2012.10.011.
11. Kishida K, Nakagawa Y, Kobayashi H, Yanagi K, Funahashi T, Shimomura I. Increased serum C1q-binding adiponectin complex to total-adiponectin ratio in men with multi-vessel coronary disease. *Diabetol Metab Syndr*. 2014;6:64. doi: 10.1186/1758-5996-6-64.
12. Ghebrehiwet B, Peerschke EI. Role of C1q and C1q receptors in the pathogenesis of systemic lupus erythematosus. *Curr Dir Autoimmun*. 2004;7:87–97.
13. Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Ann Rheum Dis*. 2012;71:1524–1529. doi: 10.1136/annrheumdis-2011-200726.
14. Magder LS, Petri M. Incidence of and risk factors for adverse cardiovascular events among patients with systemic lupus erythematosus. *Am J Epidemiol*. 2012;176:708–719. doi: 10.1093/aje/kws130.
15. Hirata A, Kishida K, Kobayashi H, Nakatsuji H, Funahashi T, Shimomura I. Correlation between serum C1q-adiponectin/total adiponectin ratio and polyvascular lesions detected by vascular ultrasonography in Japanese type 2 diabetics. *Metabolism*. 2013;62:376–385. doi: 10.1016/j.metabol.2012.08.009.
16. Bork K, Steffensen I, Machnig T. Treatment with C1-esterase inhibitor concentrate in type I or II hereditary angioedema: a systematic literature review. *Allergy Asthma Proc*. 2013;34:312–327. doi: 10.2500/aap.2013.34.3677.

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.