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Mannose binding lectin 2 haplotypes do not affect the progression of coronary atherosclerosis in men with proven coronary artery disease treated with pravastatin

Arash Alipour[a], Manuel Castro Cabezas[a,*], Jan Willem F. Elte[a], Joan-Carles Vallvé[b], Josep Ribalta[b], Aeilko H. Zwinderman[c,d,e], Joep C. Defesche[f], J. Wouter Jukema[d,e,g]

[a] Department of Internal Medicine, St. Franciscus Gasthuis Rotterdam, The Netherlands
[b] Unitat de Recerca de Lípids i Arteriosclerosi, Facultat de Medicina, Universitat Rovira i Virgili, CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Reus, Spain
[c] Department of Medical Statistics, Academic Medical Center, Amsterdam, The Netherlands
[d] Interuniversity Cardiology Institute of the Netherlands, Leiden, The Netherlands
[e] Durrer Center for Cardiogenetic Research, Utrecht, The Netherlands
[f] Department of Vascular Medicine, Academic Medical Centre, University of Amsterdam, The Netherlands
[g] Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Objective: Mannose binding lectin (MBL) is one of the three initiators of complement activation. Polymorphisms of the MBL2 gene and its promoter, and especially haplotypes, determine MBL plasma levels. MBL deficiency has been associated with the development of atherosclerosis. We evaluated whether the rate of angiographic progression of coronary atherosclerosis during pravastatin treatment was associated with MBL2 haplotypes in REGRESS, a placebo-controlled 2 years intervention study.
Methods: Three polymorphic sites in exon 1 (rs1800450, rs1800451 and rs5030737) of the MBL2 gene and 2 sites (rs7096206 and rs11003125) in the promoter region were genotyped in 398 subjects. Genotyping was performed using Applied Biosystems® TaqMan® Genotyping Assays. We divided the group in high, intermediate and low MBL2 secretor haplotypes. Quantitative coronary angiography was performed. Endpoints were mean segment diameter (MSD) and minimum obstruction diameter (MOD) established by quantitative coronary angiography.
Results: At inclusion, 50.1, 31.7 and 17.6% of the patients in the REGRESS cohort carried the high, intermediate and low MBL2 secretor haplotypes, respectively. In 0.6% of the patients, the haplotype was not informative. There were no baseline differences between the MBL2 haplotypes for age, BMI, lipid levels, leukocyte counts, CRP, MSD and MOD. The intermediate MBL2 placebo group showed the greatest increase in MSD compared to the low MBL2 group ($P = 0.03$). No difference was found for the change in MOD. No significant interaction between MBL2 haplotype groups and pravastatin therapy was observed.
Conclusion: In men with proven coronary artery disease, MBL2 secretor haplotypes are not associated to the rate of progression of coronary sclerosis nor does pravastatin treatment influence progression based on MBL2 haplotypes.

1. Introduction

Inflammation is closely associated to atherosclerosis [1,2]. In the past few years several pro-inflammatory genes have been identified, which have been suggested to play a role in atherosclerosis. One of these genes is the mannose binding lectin (MBL) gene. MBL deficiency has been associated to coronary artery disease (CAD), increased intima media thickness in carotid arteries [3–5] and atherosclerosis in different clinical situations [6–8]. Recent work from our laboratory shows that MBL deficiency may lead to a disturbed metabolism of postprandial VLDL1 lipoproteins [9], potentially favoring atherosclerosis.

MBL is an important activating factor of the lectin pathway of the complement system [10,11]. The MBL2 gene codes for the active MBL protein and has three known mutations: allele B at codon 54 (G54D, rs1800450), allele C at codon 57 (G57E, rs1800450) and allele D at codon 52 (R52C, rs5030737) [11,12]. These mutations lead to structural abnormalities and cause MBL deficiency [11]. The

* Corresponding author at: Department of Internal Medicine, Center for Diabetes and Vascular Medicine, St. Franciscus Gasthuis Rotterdam, PO Box 10900, 3004 BA Rotterdam, The Netherlands. Tel.: +31 10 4617267; fax: +31 10 4612692.
E-mail address: m.castrocabezas@sfg.nl (M.C. Cabezas).

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wild-type codon is allele A. Moreover, there are two mutations in the promoter region at −550 (H/L, rs11003125) and −221 (Y/X, rs7096206), which result in a decreased synthesis of the protein [12–14]. The alleles interact with each other to form MBL2 'secretor haplotypes' producing high, intermediate and low MBL levels [12,13].

The role of MBL in atherosclerosis is unsettled since several recent studies have suggested that not only low, but also high MBL levels are associated with atherosclerosis [15–19].

Statins have been shown to reduce the atherosclerotic burden in different groups of patients [20–22] and are able to modulate complement components in normolipidemic subjects with CAD [23]. Currently, no data are available on the role of MBL in the progression of coronary atherosclerosis in prospective intervention studies with statins. In this study, we aimed to investigate the relation between the MBL2 secretor haplotypes and the progression of coronary atherosclerosis in patients treated with pravastatin compared to placebo in the REGRESS study population [20].

2. Methods

2.1. Patients

Samples collected in the REGRESS (Regression Growth Evaluation Statin Study) cohort were used [20]. The REGRESS protocol has been described in detail elsewhere [20]. In brief, REGRESS was designed as a double-blind, placebo-controlled, multicenter study to assess the effect of pravastatin treatment on the progression and regression of coronary atherosclerosis. All patients were men of Caucasian descent; they were <70 years of age, and had angiographically documented CAD (>50% stenosis of 1 major vessel). Patients who had unstable angina or who suffered a myocardial infarction within the preceding 6 months of the study were excluded; angina pectoris classification was based on the Rose questionnaire. All patients had total cholesterol levels between 4 and 8 mmol/L and TG levels <4 mmol/L.

The present analysis was approved by the Local Ethical Committee of the Sint Franciscus Gasthuis and the Medical Ethical committee of the Leiden University Medical Center.

2.2. Analytical methods

All lipid laboratory tests were carried out at the Lipid Reference Laboratory (Atlanta, GA). Serum lipids, blood cell counts and high sensitive CRP (hsCRP) were measured in fasting blood samples by standard techniques and LDL was calculated according to the Friedewald formula [20].

2.3. Quantitative coronary arteriography

The quantitative coronary arteriographic procedures have been described in detail elsewhere and include the mean segment diameter (MSD) and minimum obstruction diameter (MOD) on a per patient basis [20].

2.4. DNA analysis and assignment of haplotype status

Genomic DNA was isolated from white cells via standard procedures, dissolved in 10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0, and stored at 4 °C. Three polymorphic sites in exon 1 (rs1800450, rs1800451, and rs5030737) of the MBL2 gene and 2 sites (rs7096206 and rs11003125) in the promoter region were genotyped in 398 subjects. Genomic DNA samples were genotyped using TaqMan® SNP Genotyping Assays specifics for each one of the polymorphic sites and according to manufacturer's instructions. The assays were performed on a GeneAmp PCR system 9700 with TaqMan® master mix (Applied Biosystems®) and allelic discrimination was done on a 7900HT fast real-time PCR system (Applied Biosystems®). To ensure consistency between runs, samples of known genotypes were repeated in every analysis.

Patients were considered to have the high MBL2 secretor haplotypes if they had the HYA and LYA haplotypes. The haplotypes LXA, HYD, HYBD and HXA were characterised as the intermediate MBL2 secretors and the haplotypes LYB and LYC as the low MBL2 secretors [12,13].

Table 1
MBL2 allele frequencies in the REGRESS cohort.

		Frequency % (no)	HWE
Promotor region polymorphism			
rs7096206	X	22.1 (156)	0.01*
	Y	77.9 (554)	
rs11003125	H	37.2 (270)	0.51
	L	62.8 (448)	
Structurally encoding polymorphism			
rs1800450	B	15.2 (111)	0.00*
	W	84.8 (448)	
rs1800451	C	3.1 (21)	0.55
	W	96.9 (655)	
rs5030737	D	9.6 (62)	0.18
	W	90.4 (584)	

HWE—Hardy–Weinberg equilibrium.
* $P < 0.05$.

3. Statistics

We first checked whether the genotype distributions of the five MBL2-gene SNPs were in Hardy–Weinberg equilibrium using one-degree of freedom Chi-square tests. Subsequently, baseline patient characteristics were compared between genotypes using ANOVA, Chi-square tests, or Kruskal–Wallis tests where appropriate. MSD and MOD changes during the trial were compared between genotypes using ANOVA with baseline values as covariates, and the occurrence of cardiac events during the trial were analyzed with the log rank test. Patients with distinctive haplotypes in the low (LYC) and intermediate (HYD, HYBD, HXA) MBL2 groups represented only a small portion of the total group. Therefore, the statistical analysis was carried out for the MBL2 secretor haplotypes, rather than the distinctive haplotypes in the three groups. We estimated individual haplotypes and the association between haplotypes and patient characteristics using phase [24] and the algorithms developed by Souverein et al. [25]. Concerning power analysis, the current sample size provided at least 80% power to detect a significant association between MBL2 genotypes and MSD/MOD change if the mean difference is larger than about 0.3 standard deviations. This was the case for rs7096206, rs11003125 and rs1800450. Because the minor allele frequencies were lower, the mean difference should be larger than 0.46 for rs5030737 and larger than 0.76 for rs1800451. The standardized mean difference (or Cohen's effect size) is considered moderate between 0.2 and 0.5 and large >0.5.

4. Results

4.1. MBL2 distribution and baseline characteristics (Tables 1–3)

The number of REGRESS patients of whom DNA was available and with at least one genotyped MBL2 SNP was 398. The relative frequency of the SNPs is listed in Table 1. Only the rs7096206 and rs1800450 SNPs were not in Hardy–Weinberg equilibrium (Table 1). The distribution of the genotypes was similar with those reported in other studies [12,13].

The MBL2 haplotype distribution was also comparable to other studies [12,13] (Table 2). The haplotype HXD with a relative fre-