



Published in final edited form as:

Stroke. 2002 May ; 33(5): 1420–1423. doi:10.1161/01.STR.0000015558.63492.B6.

## Carotid Intima-Media Thickness Is Associated With Allelic Variants of Stromelysin-1, Interleukin-6, and Hepatic Lipase Genes The Northern Manhattan Prospective Cohort Study

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### Abstract

**Background and Purpose**—Atherosclerosis is a complex disorder with hereditary and environmental causes. Carotid artery intima-media wall thickness (IMT) is a useful measure of atherosclerosis. The objective of this study was to determine the association between carotid IMT and functional promoter variants of stromelysin-1 (*MMP3*: –1612 5A>6A), interleukin-6 (*IL6*: –174G>C), and hepatic lipase (*HL*: –480C>T) genes.

**Methods**—B-mode carotid ultrasound was performed among 87 subjects (mean age, 70 ± 12 years; 55% women; 60% Caribbean-Hispanic, 25% black, and 13% white) from the Northern Manhattan Prospective Cohort Study. Carotid IMT was calculated as a composite measure (mean of the maximum IMT in the bifurcation, the common carotid artery, and the internal carotid artery).

**Results**—For all polymorphisms, genotype distribution was not significantly different from Hardy-Weinberg equilibrium. The frequencies of the rare alleles were as follows: *MMP3* –1612 5A>6A, 0.31 (95% CI, 0.25 to 0.39); *IL6* –174 G>C, 0.20 (95% CI, 0.13 to 0.25); and *HL* –480 C>T, 0.45 (95% CI, 0.35 to 0.50). Carotid IMT in the sample was 0.78±0.18 mm. Subjects with the *MMP3* genotype 6A6A had 8% greater mean carotid IMT than the other *MMP3* genotypes combined (0.95 ±0.17 versus 0.87±0.15 mm; *P*=0.04). Subjects with the *IL6* genotype GG had 11% greater IMT (0.85±0.17 versus 0.76±0.16 mm; *P*=0.03), and those with the *HL* genotype CC had 13% greater IMT (0.87±0.20 versus 0.76±0.18 mm; *P*=0.02) than the other genotypes combined. Adjustment for other risk factors did not change these associations.

**Conclusions**—Carotid IMT is higher among subjects homozygous for functional variants in genes related to matrix deposition (*MMP3* –16126A), inflammation (*IL6* –174G), and lipid metabolism (*HL* –480C). These associations were independent of race-ethnicity and some environmental exposures. Further studies are needed to confirm these genotype-phenotype associations.

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Presented in part at the 26th American Heart Association International Conference on Stroke and Cerebral Circulation, Ft Lauderdale, Fla, February 14–16, 2001.

## Keywords

genetics; interleukin-6; intima-media thickness; lipase; stromelysin 1; ultrasonography

Atherosclerosis is a common, but complex, multifactorial disorder that accounts for much of morbidity and mortality in adult life. Carotid artery intima-media thickness (IMT) measured by high-resolution B-mode ultrasound has been demonstrated to correlate well with pathologically and clinically defined atherosclerosis<sup>1,2</sup> and increased risk of myocardial infarction and stroke.<sup>3</sup>

Several complex mechanisms have been hypothesized to play a key role in the development of atherosclerosis, including metabolic and inflammatory processes. There is some evidence that variants of specific genes are associated with these processes. A variant in the promoter of the stromelysin-1 (*MMP3*) gene [-1612(5A/6A)] regulates transcription of stromelysin-1, one of the matrix metalloproteinases involved in connective tissue remodeling, an essential part of the atherosclerotic process.<sup>4,5</sup> This variant has been associated with carotid IMT and progression of angiographically determined coronary artery disease.<sup>4,5</sup> A polymorphism of the interleukin-6 (*IL6*) gene with a G>C change at position -174 has been recently identified.<sup>6</sup> It has been reported that individuals homozygous for the G allele have greater IMT than other subjects.<sup>4</sup> For the hepatic lipase (*HL*) gene, a number of polymorphisms have been reported in the promoter, all showing strong allelic association.<sup>7</sup> The C>T variation at -480 results in low *HL* activity and higher HDL levels.<sup>8</sup> No study has yet reported on the impact of the *HL* promoter variants on carotid IMT.

The aim of this study was to investigate the association between carotid IMT and promoter variants of stromelysin-1, interleukin-6, and hepatic lipase genes.

## Subjects and Methods

The study population of 87 stroke-free subjects was randomly derived from the Northern Manhattan Prospective Cohort Study.<sup>9</sup> The mean age was 70±12 years; 55% were women; 60% were Caribbean-Hispanic, 25% black, and 13% white.

Carotid IMT was assessed by high-resolution B-mode ultrasound (Diasonics 2D-Gateway, 7-MHz probe) with the use of the Atherosclerosis Risk in Communities (ARIC) protocol.<sup>2</sup> The sonographers and the readers were certified for this protocol. Interreader and intrareader reliability values for carotid IMT in our laboratory were similar to those published in other studies.<sup>2,3</sup> In a sample of 88 stroke-free community subjects, mean absolute difference of carotid IMT between 2 readers was 0.19±0.36 mm, with a variation coefficient of 7.5%, a correlation coefficient of 0.77, and a percent error of 10.6%. Intrareader mean absolute IMT difference was 0.07±0.04 mm, with a variation coefficient of 5.4%, a correlation coefficient of 0.94, and a percent error of 5.6%.

The carotid artery IMT protocol consisted of scanning the carotid arteries in 3 segments, defined as follows (Figure 1): (1) near wall and far wall of the segment extending from 10 to 20 mm proximal to the tip of the flow divider into the common carotid artery (CCA); (2) near wall and far wall of the carotid bifurcation beginning at the tip of the flow divider and extending 10 mm proximal to the flow divider tip; and (3) near wall and far wall of the proximal 10 mm of the internal carotid artery (ICA). Measurements of IMT were performed off-line with the use of IMAGE-Pro (Microsoft) image analysis software.

The carotid IMT was calculated as a composite measure (mean of the 12 sites) that combined the near and the far wall of the maximal CCA IMT, the maximal bifurcation IMT, and the maximal ICA IMT of both sides.

### Genetic Analysis

Blood samples taken at the time of IMT examination were stored at  $-20^{\circ}\text{C}$  until extraction of DNA with the use of the Signal Sequence Trap method, as described.<sup>10</sup> DNA was transported to University College London (London, UK) for genotyping. Detailed descriptions of the methods used to determine the genotypes examined in this study have been provided previously.<sup>4,6,11–13</sup>

### Statistical Analysis

The frequencies of alleles of the specific candidate genes were compared between various race-ethnicity groups by  $\chi^2$  test with Bonferroni correction. The association of gene polymorphisms and carotid IMT was analyzed with a multiple linear regression model (SAS version 6.12) adjusted for demographics, hypertension, cholesterol, and current smoking.

### Results

Despite the small numbers of subjects, the genotype distributions were consistent with that expected from Hardy-Weinberg equilibrium (Table 1). For *MMP3* gene among blacks, 82% were homozygous for 6A6A in comparison to 46% of whites and 39% of Caribbean-Hispanics ( $P<0.05$ ), although there was no significant evidence of heterogeneity of allele frequency. For *IL6*, as reported previously,<sup>6</sup> the frequency of the  $-174\text{C}$  allele among blacks was slightly lower than among other ethnicities ( $P=0.09$ ). However, 92% of blacks were homozygous for GG of the *IL6* gene, in comparison to 66% of Caribbean-Hispanics and 42% of whites ( $P<0.05$ ). For *HL*, the  $-480\text{C}$  allele frequency was lowest in whites, but overall there was no evidence for heterogeneity of frequency. For the *HL* gene, however, 73% of whites but only 27% of Caribbean-Hispanics and 25% of blacks were homozygous for CC of the *HL* gene ( $P<0.05$ ). The frequencies of the rare alleles were as follows: *MMP3*  $-1612\ 5\text{A}>6\text{A}$ , 0.31 (95% CI, 0.25 to 0.39); *IL6*  $-174\ \text{G}>\text{C}$ , 0.20 (95% CI, 0.13 to 0.25); and *HL*  $-480\ \text{C}>\text{T}$ , 0.45 (95% CI, 0.35 to 0.50). For *MMP3* and *IL6*, but not *HL*, these frequencies were significantly lower than previously reported in white subjects.<sup>4</sup>

### Gene Polymorphisms and Carotid IMT

The genotypes with a minor allele were combined to increase statistical power (the 2 genotypes with a minor allele were not significantly different, as seen in Table 2). Subjects with the genotypes *MMP3* 6A6A (Figure 2) had 8% greater mean carotid IMT than the other genotypes combined ( $0.95\pm 0.17$  versus  $0.87\pm 0.15$  mm;  $P=0.044$ ). Subjects with *IL6* GG had 11% greater IMT ( $0.85\pm 0.17$  versus  $0.76\pm 0.16$  mm;  $P=0.034$ ), and those with *HL* CC had 13% greater IMT ( $0.87\pm 0.20$  versus  $0.76\pm 0.18$  mm;  $P=0.025$ ). After adjustment these associations remained significant, each explaining 19% of the variance in carotid IMT.

### Discussion

We observed that subjects homozygous for specific candidate genes related to matrix deposition (*MMP3* gene), inflammation (*IL6* gene), and lipid metabolism (*HL* gene) had an increased carotid artery wall thickness. Although the biology of the vessel wall and development of atherosclerosis are most likely regulated by multiple genes, the investigated genes may explain some aspects of vascular physiology. All known environmental risk factors account for only approximately half of all cases of atherosclerotic diseases.<sup>14</sup> The rest may be

explained by some newly recognized genetic factors, including specific gene polymorphisms investigated in our study.

Stromelysin-1, as a key regulator of matrix remodeling, may be involved in development of atherosclerosis. Individuals who produce less *MMP3* would have a lower ability to remodel and degrade the matrix components, leading to faster arterial wall thickening and plaque growth.<sup>4,5</sup> We observed that 6A homozygous subjects had an 8% increase in carotid IMT compared with other genotypes combined. This strongly confirms the association between the 6A6A genotype and higher carotid IMT reported in Finnish men, in whom IMT was 15% higher in this group than in other genotypes.<sup>4</sup> The inflammatory response may be modulated by polymorphisms in the promoter of the *IL6* gene. We observed that carotid IMT was 11% greater among subjects homozygous for the G allele in comparison to other genotypes, confirming previous results.<sup>4</sup> Genetic polymorphism in the promoter region of the *HL* gene plays a major role in the control of HDL<sub>2</sub> cholesterol.<sup>15</sup> High *HL* activity is associated with an increase in small dense LDL particles and increased risk of vascular events.<sup>8</sup> We observed that individuals homozygous for the C allele of the *HL* gene had 13% higher carotid IMT than other genotypes.

In summary, our study showed a genetic effect on carotid IMT in the 3 candidate genes despite small sample size, which did not allow for the analysis of combined genotypes or test for gene×environment interaction. Increased carotid IMT may be an important link between genetic predisposition, environmental exposures, and stroke. Our study confirms the associations seen between the *MMP3* and *IL6* gene variants and carotid IMT in Finnish men<sup>4</sup> and extends these associations to Hispanic subjects, who made up the majority of this sample. This is, according to our knowledge, the first report on association between the *HL* gene promoter variants and carotid IMT. Further studies with increased power are needed to confirm these genotype-phenotype associations.

## Acknowledgments

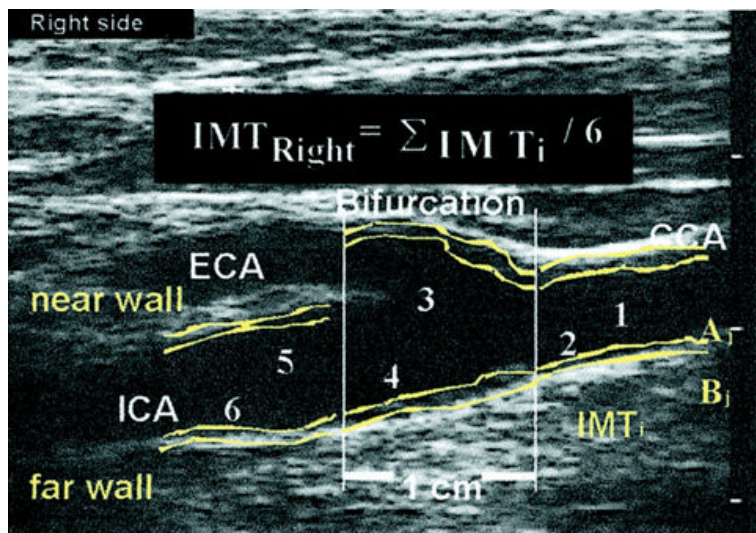
Tanja Rundek is supported by the Hazel K. Goddess Fund for Stroke Research in Women.

Mitchell S. Elkind is supported by Centers for Disease Control Cooperative Agreement U50/CCU216543. John Pittman, Bernadette Boden-Albala, and Ralph L. Sacco are supported by National Institutes of Health/National Institute of Neurological Disorders and Stroke grant NS-R01-29993. Suh-Hang Hank Juo is supported by National Institutes of Health/National Human Genome Research Institute grant IPA-5-20562. Steve Martin and Steve E. Humphries are supported by the British Heart Foundation (RG2000015). We would like to thank Dr J.P. Mohr for providing access to the ultrasound laboratory and supporting carotid atherosclerosis research. We also thank Drs Romel Ramas, Sam Trocio, and Oscar Ramos for performing the ultrasound scans.

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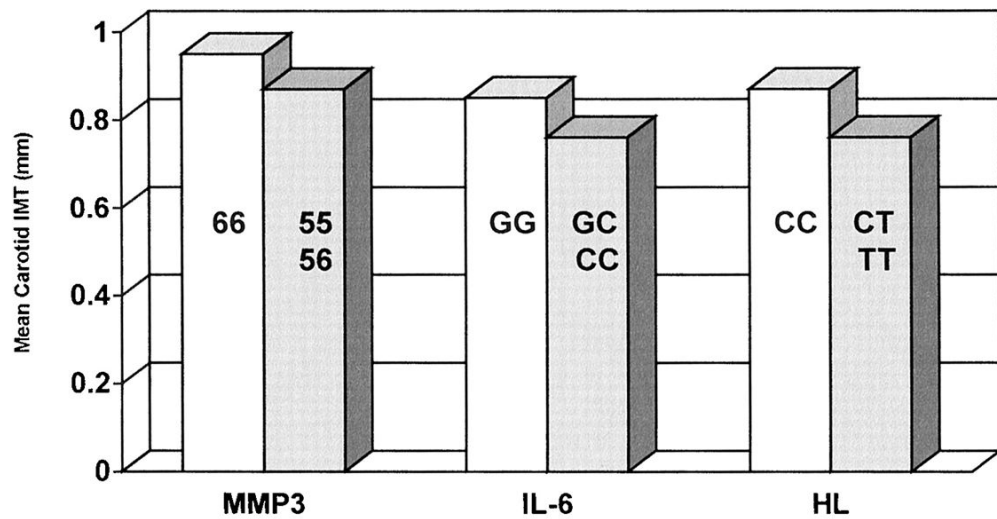


$$IMT = \sum_{i=1}^{12} IMT_i / 12$$

$$IMT_i = \text{Max}_j (A_j - B_j)$$

**Figure 1.** Measurement of carotid IMT. ICA indicates internal carotid artery; ECA, external carotid artery; and CCA, common carotid artery. Max<sub>j</sub> is maximum distance between 2 lines measured in j points of define segment.





$\Delta$ IMT ( $IMT_1 - IMT_2$ ) / $IMT_2 \times 100$	8%	11%	13%
<b>P*</b>	<b>0.043</b>	<b>0.048</b>	<b>0.027</b>
Parameter estimate for separate gene	0.245	0.235	0.329
$R^2$	0.188	0.195	0.193

**Figure 2.** Carotid IMT and *MMP3*, *IL6*, and *HL* gene polymorphism. \*Multiple linear regression model adjusted for race-ethnicity, hypertension, cholesterol, and current smoking.

**TABLE 1**  
Distributions of Genotypes of Stromelysin-1 (*MMP3*), Interleukin-6 (*IL6*), and Hepatic Lipase (*HL*) Genes With Respect to Race-Ethnicity Among 87 Stroke-Free Subjects

Gene and Polymorphism	Overall	Caribbean-Hispanic	Black	White
Stromelysin-1 ( <i>MMP3</i> ) <sup>*</sup>				
5A5A	7 (11)	3 (7)	1 (9)	3 (27)
5A6A	27 (41)	22 (54)	1 (9)	3 (27)
6A6A	32 (48)	16 (39)	9 (82)	5 (46)
Frequency of 6A allele	0.69 (0.61–0.77)	0.66 (0.56–0.76)	0.86 (0.72–1.00)	0.59 (0.39–0.80)
Interleukin-6 ( <i>IL6</i> ) <sup>†</sup>				
GG	47 (66)	29 (66)	11 (92)	5 (42)
GC	18 (26)	12 (27)	1 (8)	5 (42)
CC	5 (8)	3 (7)	0 (0)	2 (16)
Frequency of C allele	0.20 (0.13–0.27)	0.21 (0.12–0.29)	0.04 (0.00–0.12)	0.38 (0.18–0.57)
Hepatic lipase ( <i>HL</i> ) <sup>‡</sup>				
CC	23 (34)	11 (27)	3 (25)	8 (73)
CT	28 (42)	8 (44)	7 (58)	2 (18)
TT	16 (24)	12 (29)	2 (17)	1 (9)
Frequency of T allele	0.45 (0.36–0.53)	0.51 (0.39–0.64)	0.46 (0.26–0.66)	0.18 (0.02–0.34)

Values are number (percent) or (95% CI). Denominators vary because not all subjects had genotypes determined for all gene polymorphisms.

<sup>\*</sup> $\chi^2=11.31$ ,  $P<0.05$ .

<sup>†</sup> $\chi^2=7.14$ ,  $P<0.05$ .

<sup>‡</sup> $\chi^2=10.64$ ,  $P<0.05$ .



**TABLE 2**  
Association of Carotid IMT and Alleles of *MMP3*, *IL6*, and *HL* Genes

Gene and Polymorphism	n (%)	Mean Carotid IMT, mm	<i>t</i>	<i>P</i> *
Stromelysin-1 ( <i>MMP3</i> )				
5A5A	8 (12)	0.8130.15		
5A6A	27 (40)	0.8930.20		
6A6A*	32 (48)	0.9530.17	2.046	0.044
Interleukin-6 ( <i>IL6</i> )				
CC	5 (7)	0.7230.15		
GC	19 (27)	0.7830.21		
GG*	47 (66)	0.8530.17	2.152	0.034
Hepatic lipase ( <i>HL</i> )				
TT	16 (24)	0.6930.19		
CT	28 (42)	0.7730.17		
CC*	23 (34)	0.8730.20	2.286	0.025

\* *P* values correspond to the comparisons of IMT means between 6A6A vs 5A6A/5A5A ( $0.95\pm 0.17$  vs  $0.87\pm 0.15$ ); GG vs GC/CC ( $0.85\pm 0.17$  vs  $0.76\pm 0.16$ ); CC vs CT/TT ( $0.87\pm 0.20$  vs  $0.76\pm 0.18$ ).