Supplemental Material

Prevalence and determinants of carotid plaque in the REFINE-Reykjavik study – a cross sectional study

Ran Sturlaugsdottir MSc[a,b],

Thor Aspelund PhD[a,b], Gudlaug Bjornsdottir MSc[a, b], Sigurdur Sigurdsson MSc[a], Bolli Thorsson MD[a], Gudny Eiriksdottir MSc[a], Vilmundur Gudnason MD, PhD[a,b]

[a]Icelandic Heart Association, Kopavogur, Iceland.

[b]University of Iceland, Reykjavik, Iceland.

Address for Correspondence:

Vilmundur Gudnason MD, PhD, Director of the Icelandic Heart Association Research Institute

Holtasmari 1, 201 Kopavogur

Tel: 354-535 1800

Fax: 354-535 1801

Email: v.gudnason@hjarta.is

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Supplemental methods

The REFINE-Reykjavik study uses strict quality control procedures for monitoring and testing consistency in image acquisition and image analysis. The quality control includes periodical tests of image analysis and acquisition reproducibility including re-reading of IMT every 6 months of the same 24 cases for assessment of inter-and intra-observer variability and consistency over time. There were typically 2 weeks between reading 1 and reading 2 for the intra-observer variability assessment. Inter-observer variability of carotid plaque presence and severity was tested by repeated acquisitions of up to 15 studies every year by each sonographer. In addition, intra-observer variability of IMT was further tested by the re-reading of 10 randomly selected studies by each observer every 6 months where there were typically 5 to 6 months between reading 1 and reading 2.

Mean intra-observer variability in IMT measurements for three observers (intra-class correlation and percent coefficient of variation respectively) based on the re-reading of the same 24 cases (n=24) over the course of the study ranged from 0.97 to 0.99 and 2.7% to 3.6% for the far wall of the carotid arteries and 0.96 to 0.97 and 3.6% to 4.9% for the near wall. Inter-observer variability for the same 24 cases and the same observers ranged from 0.91 to 0.94 and 4.7% to 6.0% for the far wall and 0.79 to 0.81 and 8.4% to 9.2% for the near wall. Intra-reliability assessment (kappa statistics) of carotid plaque presence and plaque severity between the observers where the results by two observers were compared to the results of one observer that was considered a gold standard were 0.77 (n=68) and 0.84 (n=60) demonstrating good to excellent agreement. The intra-observer variability in IMT measurements based on re-reading of

a random selection of 10 cases every 6 months (intra-class correlation and percent coefficient of variation respectively) was 0.96 and 3.7% for the far wall and 0.91 and 5.6% for the near wall for observer 1 (accumulative total of re-readings, n=90), 0.93 and 5.0% for the far wall and 0.92 and 6.3% for the near wall for observer 2 (accumulative total of re-readings, n=80) and 0.94 and 3.2% for the far wall and 0.96 and 3.5% for the near wall for observer 3 (accumulative total of re-readings, n=50).

Supplemental Tables

Supplemental Table 1: Carotid plaque prevalence and common carotid artery-IMT (CCA-IMT) in age groups for the total sample, men and women. Data are presented as number (%) for carotid plaque and mean (SD) for CCA-IMT.

| | Number in age group (n) | Carotid plaque category | | | | CCA-IMT |
		No	Minimal	Moderate	Severe	
Total sample						
25-29	336	321 (95.5)	15 (4.5)	0 (0)	0 (0)	0.55 (0.06)
30-34	366	327 (89.3)	36 (9.8)	3 (0.8)	0 (0)	0.59 (0.07)
35-39	582	516 (88.7)	63 (10.8)	3 (0.5)	0 (0)	0.62 (0.08)
40-44	915	665 (72.7)	231 (25.3)	19 (2.1)	0 (0)	0.66 (0.08)
45-49	891	556 (62.4)	297 (33.3)	36 (4.0)	2 (0.2)	0.70 (0.09)
50-54	962	479 (49.8)	418 (43.5)	63 (6.6)	2 (0.2)	0.75 (0.10)
55-59	932	354 (38.0)	441 (47.3)	126 (13.5)	11 (1.2)	0.81 (0.12)
60-64	920	259 (28.2)	461 (50.1)	174 (18.9)	26 (2.8)	0.85 (0.12)
65-69	620	116 (18.7)	318 (51.3)	155 (25.0)	31 (5.0)	0.88 (0.13)
Total	6524	3593 (55.1)	2280 (34.9)	579 (8.9)	72 (1.1)	0.73 (0.14)
Men						
25-29	169	160 (94.7)	9 (5.3)	0 (0)	0 (0)	0.57 (0.07)
30-34	177	152 (85.9)	23 (13.0)	2 (1.1)	0 (0)	0.60 (0.08)
35-39	285	253 (88.8)	31 (10.9)	1 (0.4)	0 (0)	0.64 (0.09)
40-44	446	306 (68.6)	128 (28.7)	12 (2.7)	0 (0)	0.68 (0.08)
45-49	445	257 (57.8)	168 (37.8)	19 (4.3)	1 (0.2)	0.72 (0.09)
50-54	444	192 (43.2)	216 (48.7)	35 (7.9)	1 (0.2)	0.78 (0.11)
55-59	465	139 (29.9)	240 (51.6)	79 (17.0)	7 (1.5)	0.84 (0.12)
60-64	461	106 (23.0)	236 (51.2)	105 (22. 8)	14 (3.0)	0.87 (0.13)
65-69	312	49 (15.7)	160 (51.3)	87 (27.9)	16 (5.1)	0.91 (0.13)
Total	3204	1614 (50.4)	1211 (37.8)	340 (10.6)	39 (1.2)	0.76 (0.15
Women						
25-29	167	161 (96.4)	6 (3.6)	0 (0)	0 (0)	0.53 (0.05)***
30-34	189	175 (92.6)	13 (6.9)*	1 (0.5)	0 (0)	0.57 (0.06)***
35-39	297	263 (88.6)	32 (10.8)	2 (0.7)	0 (0)	0.60 (0.07)***
40-44	469	359 (76.6)	103 (22.0)*	7 (1.5)	0 (0)	0.64 (0.07)***
45-49	446	299 (67.0)	129 (28.9)**	17 (3.8)	1 (0.2)	0.68 (0.08)***
50-54	518	287 (55.4)	202 (39.0)**	28 (5.4)	1 (0.2)	0.74 (0.09)***
55-59	467	215 (46.0)	201 (43.0)**	47 (10.1)**	4 (0.9)	0.78 (0.10)***
60-64	459	153 (33.3)	225 (49.02)	69 (15.03)**	12 (2.6)	0.83 (0.11)***
65-69	308	67 (21.8)	158 (51.3)	68 (22.08)	15 (4.9)	0.85 (0.11)***

| Total | 3320 | 1979 (59.6) | 1069 (32.2)*** | 239 (7.2)*** | 33 (1) | 0.71 (0.13)*** |

*p<0.05; **p<0.01 and *** p<0.001 refer to sex difference.

CCA-IMT=common carotid intima-media thickness

Supplemental Table 2: Carotid plaque association with cardiovascular risk factors (multinomial logistic regression analysis) in sex

specific analysis.

.	Men					Women				
	Model 1			Model 2		Model 1			Model 2	
Moderate plaque (vs. no plaque)	OR (95%CL)	P		OR (95%CL)	P	OR (95%CL)	P		OR (95%CL)	P
Age, 5 years				2.12 (1.92-2.34)	<0.001				1.82 (1.63-2.04)	<0.001
TC, mmol/l	1.15 (1.02-1.31)	<0.05		1.51 (1.28-1.78)	<0.001	1.13 (0.99-1.30)	0.08		1.30 (1.10-1.55)	<0.01
Log TG, mmol/l	1.57 (1.21-2.03)	<0.001		0.96 (0.69-1.33)	0.80	2.38 (1.79-3.18)	<0.001		1.12 (0.76-1.65)	0.59
Log CRP, mg/l	1.31 (1.15-1.49)	<0.001		1.18 (1.01-1.38)	<0.05	1.25 (1.09-1.42)	<0.01		1.12 (0.95-1.33)	0.18
BMI, 5 units	0.87 (0.75-1.01)	0.06		0.61 (0.51-0.74)	<0.001	1.07 (0.94-1.22)	0.30		0.81 (0.68-0.96)	<0.05
SBP, 10 mmHg	1.26 (1.16-1.36)	<0.001		1.28 (1.17-1.40)	<0.001	1.46 (1.35-1.59)	<0.001		1.46 (1.33-1.61)	<0.001
Former smoker (vs. never smoked)	2.19 (1.59-3.01)	<0.001		1.88 (1.32-2.68)	<0.001	1.78 (1.26-2.51)	<0.01		1.61 (1.11-2.33)	<0.05
Current smoker (vs. never smoked)	4.51 (3.14-6.49)	<0.001		3.47 (2.28-5.27)	<0.001	5.24 (3.61-7.62)	<0.001		4.23 (2.76-6.47)	<0.001
T2DM	2.80 (1.77-4.41)	<0.001		2.48 (1.47-4.18)	<0.001	4.11 (2.20-7.70)	<0.001		2.23 (1.08-4.58)	<0.05
Family MI	1.81 (1.40-2.35)	<0.001		1.50 (1.13-2.00)	<0.01	1.45 (1.09-1.91)	<0.05		1.20 (0.88-1.63)	0.25
Physically active	0.65 (0.50-0.85)	<0.01		0.87 (0.64-1.19)	ns	0.60 (0.45-0.79)	<0.001		0.88 (0.64-1.21)	0.44
Elementary school (vs. university education)	3.22 (2.16-4.79)	<0.001		2.69 (1.72-4.21)	<0.001	2.93 (1.98-4.32)	<0.001		1.70 (1.10-2.63)	<0.05
High school (vs. university education)	2.09 (1.53-2.87)	<0.001		1.76 (1.23-2.51)	<0.01	1.82 (1.21-2.73)	<0.01		1.34 (0.86-2.10)	0.20
Junior college (vs. university education)	1.61 (0.97-2.67)	ns		1.56 (0.89-2.73)	ns	1.22 (0.67-2.21)	ns		0.92 (0.48-1.75)	ns
Statin use	2.68 (1.92-3.76)	<0.001		2.17 (1.36-3.45)	<0.01	3.77 (2.45-5.80)	<0.001		2.85 (1.64-4.95)	<0.001
Antihypertensive medication use	2.24 (1.69-2.97)	<0.001		1.85 (1.31-2.61)	<0.001	2.31 (1.72-3.11)	<0.001		1.79 (1.26-2.53)	<0.01
CHD	3.48 (2.14-5.68)	<0.001		2.82 (1.55-5.12)	<0.001	4.40 (1.99-9.73)	<0.001		2.11 (0.87-5.10)	0.10
Minimal plaque (vs. no plaque)										
Age, 5 years				1.54 (1.47-1.62)	<0.001				1.44 (1.37-1.53)	<0.001
TC, mmol/l	1.37 (1.26-1.49)	<0.001		1.38 (1.24-1.53)	<0.001	1.18 (1.09-1.29)	<0.001		1.14 (1.03-1.27)	<0.05
Log TG, mmol/l	1.65 (1.40-1.95)	<0.001		1.23 (1.00-1.51)	<0.05	1.61 (1.36-1.92)	<0.001		1.24 (0.99-1.57)	0.07
Log CRP, mg/l	1.18 (1.08-1.29)	<0.001		1.16 (1.05-1.29)	<0.01	1.05 (0.97-1.14)	ns		0.93 (0.85-1.03)	ns
BMI, 5 units	0.97 (0.88-1.06)	0.47		0.76 (0.68-0.86)	<0.001	1.03 (0.95-1.12)	ns		0.93 (0.84-1.03)	ns
SBP, 10 mmHg	1.19 (1.12-1.26)	<0.001		1.17 (1.10-1.25)	<0.001	1.24 (1.18-1.32)	<0.001		1.25 (1.17-1.33)	<0.001
Former smoker (vs. never smoked)	1.25 (1.03-1.51)	<0.05		1.14 (0.93-1.40)	0.20	1.19 (0.99-1.43)	0.07		1.25 (1.02-1.53)	<0.05
Current smoker (vs. never smoked)	1.71 (1.36-2.14)	<0.001		1.47 (1.14-1.89)	<0.01	1.88 (1.51-2.35)	<0.001		1.90 (1.47-2.45)	<0.001

T2DM	1.67 (1.13-2.46)	<0.05		1.45 (0.94-2.22)	0.09	1.90 (1.12-3.24)	<0.05	1.53 (0.85-2.79)	0.16
Family MI	1.22 (1.02-1.45)	<0.05		1.14 (0.94-1.37)	0.19	1.28 (1.08-1.52)	<0.01	1.20 (1.00-1.44)	<0.05
Physically active	0.88 (0.74-1.04)	ns		1.09 (0.90-1.32)	ns	0.87 (0.74-1.03)	ns	1.04 (0.86-1.25)	ns
Elementary school (vs. university education)	1.51 (1.16-1.96)	<0.01		1.41 (1.06-1.88)	<0.05	1.29 (1.04-1.60)	<0.05	1.04 (0.81-1.32)	0.77
High school (vs. university education)	1.25 (1.03-1.52)	<0.05		1.18 (0.95-1.46)	0.13	1.11 (0.90-1.37)	ns	0.96 (0.76-1.22)	ns
Junior college (vs. university education)	1.35 (1.02-1.79)	<0.05		1.32 (0.98-1.79)	0.07	1.13 (0.86-1.48)	ns	0.92 (0.68-1.24)	ns
Statin use	1.38 (1.03-1.84)	<0.05		1.74 (1.21-2.50)	<0.01	1.39 (0.95-2.04)	ns	1.26 (0.81-1.98)	ns
Antihypertensive medication use	1.40 (1.13-1.74)	<0.01		1.34 (1.05-1.72)	<0.05	1.46 (1.20-1.78)	<0.001	1.29 (1.02-1.62)	<0.05
CHD	1.07 (0.66-1.71)	ns		0.99 (0.57-1.70)	ns	1.34 (0.62-2.89)	ns	0.96 (0.42-2.18)	ns

Model 1: age adjusted analysis.

Model 2: multivariable adjusted analysis including age, sex, TC, log TG, log CRP, BMI, SBP, smoking status, T2DM, family MI, physical activity, education level, statin use,
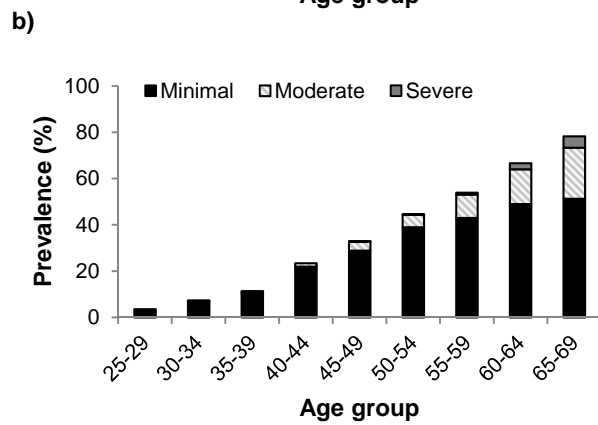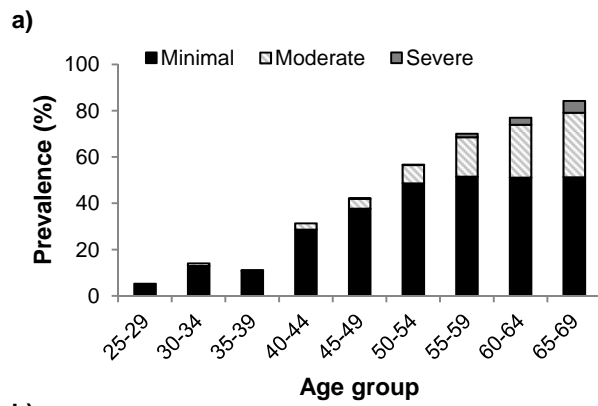
antihypertensive medication use, CHD.

Supplemental Table 3: Common carotid artery-IMT association with cardiovascular risk factors in men and women.

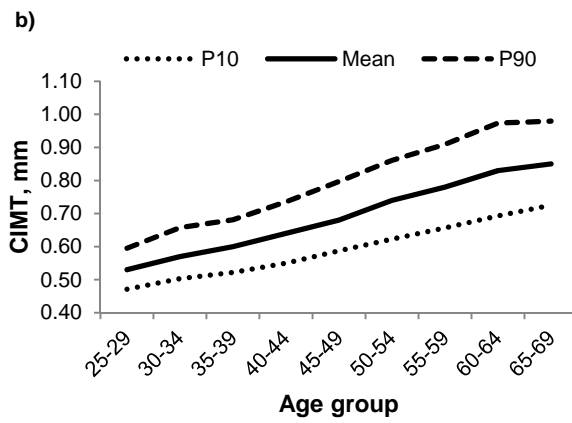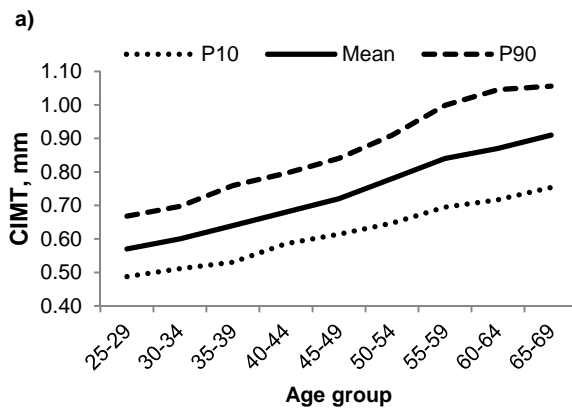|	Men					Women				
	Model 1			Model 2		Model 1			Model 2	
	Estimate (SE)	P		Estimate (SE)	P	Estimate (SE)	P		Estimate (SE)	P
Age, 5 years	-	-		0.042 (0.001)	<0.001	-	-		0.037 (0.001)	<0.001
TC, mmol/l	0.002 (0.002)	ns		0.003 (0.002)	ns	0.007 (0.002)	<0.001		0.006 (0.002)	<0.001
Log TG, mmol/l	0.019 (0.004)	<0.001		0.001 (0.004)	0.97	0.017 (0.003)	<0.001		-0.003 (0.004)	0.40
Log CRP, mg/l	0.009 (0.002)	<0.001		0.001 (0.002)	0.53	0.006 (0.001)	<0.001		0.000 (0.002)	0.82
BMI, 5 units	0.020 (0.002)	<0.001		0.015 (0.002)	<0.001	0.011 (0.001)	<0.001		0.009 (0.002)	<0.001
SBP, 10 mmHg	0.014 (0.001)	<0.001		0.012 (0.001)	<0.001	0.014 (0.001)	<0.001		0.013 (0.001)	<0.001
Former smoker (vs. never smoked)	0.023 (0.004)	<0.001		0.016 (0.004)	<0.001	0.011 (0.004)	<0.01		0.011 (0.003)	<0.01
Current smoker (vs. never smoked)	0.023 (0.005)	<0.001		0.021 (0.005)	<0.001	0.015 (0.004)	<0.001		0.018 (0.004)	<0.001
T2DM	0.034 (0.008)	<0.001		0.010 (0.008)	0.21	0.016 (0.009)	ns		-0.001 (0.009)	ns
Family MI	0.006 (0.004)	ns		0.002 (0.004)	ns	0.001 (0.003)	ns		-0.003 (0.003)	ns
Physically active	-0.005 (0.004)	ns		0.006 (0.004)	ns	-0.001 (0.003)	ns		0.006 (0.003)	ns
Elementary school (vs. university education)	0.033 (0.006)	<0.001		0.024 (0.006)	<0.001	0.009 (0.004)	<0.05		0.000 (0.004)	0.94
High school (vs. university education)	0.016 (0.004)	<0.001		0.009 (0.004)	<0.05	0.003 (0.004)	ns		-0.002 (0.004)	ns
Junior college (vs. university education)	0.000 (0.006)	ns		-0.003 (0.006)	ns	0.006 (0.005)	ns		0.001 (0.005)	ns
Statin use	0.017 (0.006)	<0.01		0.011 (0.007)	0.14	0.014 (0.007)	<0.05		0.018 (0.008)	<0.05
Antihypertensive medication use	0.008 (0.005)	0.10		-0.013 (0.005)	<0.01	0.007 (0.004)	ns		-0.004 (0.004)	ns
CHD	0.031 (0.009)	<0.001		0.030 (0.010)	<0.01	-0.001 (0.013)	ns		-0.009 (0.013)	ns

Model 1: age adjusted analysis.

Model 2: multivariable adjusted analysis including age, sex, TC, log TG, log CRP, BMI, SBP, smoking status, T2DM, family MI, physical activity, education level, statin use, antihypertensive medication use, CHD.

Supplemental Figures

a)

b)

Supplemental Figure 1: Prevalence of minimal, moderate and severe carotid plaque by age in men (a) and women (b).

Supplemental Figure 2: Mean common carotid artery-IMT and 90th and 10th percentiles by age in men (a) and women (b).