ORIGINAL RESEARCH

A Low-Frequency Variant in *MAPK14* Provides Mechanistic Evidence of a Link With Myeloperoxidase: A Prognostic Cardiovascular Risk Marker

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***Background*—**Genetics can be used to predict drug effects and generate hypotheses around alternative indications. To support Losmapimod, a p38 mitogen-activated protein kinase inhibitor in development for acute coronary syndrome, we characterized gene variation in *MAPK11/14* genes by exome sequencing and follow-up genotyping or imputation in participants well-phenotyped for cardiovascular and metabolic traits.

***Methods and Results*—**Investigation of genetic variation in *MAPK11* and *MAPK14* genes using additive genetic models in linear or logistic regression with cardiovascular, metabolic, and biomarker phenotypes highlighted an association of RS2859144 in *MAPK14* with myeloperoxidase in a dyslipidemic population (Genetic Epidemiology of Metabolic Syndrome Study), $P=2.3\times10^{-6}$). This variant (or proxy) was consistently associated with myeloperoxidase in the Framingham Heart Study and Cardiovascular Health Study studies (replication meta-$P=0.003$), leading to a meta-P value of 9.96×10^{-7} in the 3 dyslipidemic groups. The variant or its proxy was then profiled in additional population-based cohorts (up to a total of 58 930 subjects) including Cohorte Lausannoise, Ely, Fenland, European Prospective Investigation of Cancer, London Life Sciences Prospective Population Study, and the Genetics of Obesity Associations study obesity case–control for up to 40 cardiovascular and metabolic traits. Overall analysis identified the same single nucleotide polymorphisms to be nominally associated consistently with glomerular filtration rate ($P=0.002$) and risk of obesity (body mass index ≥30 kg/m^2, $P=0.004$).

***Conclusions*—**As myeloperoxidase is a prognostic marker of coronary events, the *MAPK14* variant may provide a mechanistic link between p38 map kinase and these events, providing information consistent with current indication of Losmapimod for acute coronary syndrome. If replicated, the association with glomerular filtration rate, along with previous biological findings, also provides support for kidney diseases as alternative indications. **(*J Am Heart Assoc*. 2014;3:e001074 doi: 10.1161/JAHA.114.001074)**

Key Words: acute coronary syndrome • drug target gene • exome sequencing • myeloperoxidase • rare variation

Previously, Losmapimod (a fast-acting p38 MAPK-α and MAPK-β inhibitor) and related compounds had been applied toward a number of indications, such as rheumatoid arthritis and depression, with failure to obtain proof-of-concept. These chronic disease settings may not take advantage of the rapid stress-mediated response inherent in p38 mitogen-activated protein kinase (MAPK) activity. MAPK is an intracellular kinase that functions as an important

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This article was handled independently by Julie Johnson, PharmD, as a guest editor. The editors had no role in the evaluation of the manuscript or in the decision about its acceptance.

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Received June 11, 2014; accepted July 23, 2014.

mediator of the inflammatory signaling cascade that leads to activation of cytokine production, more easily observed during acute events, such as acute coronary syndrome. In the setting of statin therapy, Losmapimod significantly attenuated the postpercutaneous intervention inflammatory response, as measured by high-sensitivity C-reactive protein (CRP).[1] In a phase 2 study, the effects of Losmapimod on safety, infarct size, and cardiac function were evaluated.[2] Encouraging results[3] have led to the progression of Losmapimod to a phase 3 outcome trial for acute coronary syndromes.

The genes that encode MAPK-α and -β (*MAPK14* and *MAPK11*, respectively) were included in a large sequencing experiment examining the exons and flanking regions of 202 drug target genes in more than 14 000 participants phenotyped for a wide range of diseases and medically relevant traits.[4] The Nelson et al sequencing study reported common and rare variation in 202 genes including *MAPK14* and *MAPK11*, drug target genes for Losmapimod, and tested associations of common variants and rare coding variants, in aggregate, with 12 diseases.[4] No common diseases were found to be associated with *MAPK14* or *MAPK11* in any published study including Nelson et al.[4] The present work focuses on profiling variants within the *MAPK11* and *MAPK14* genes for association with cardiovascular and metabolic phenotypes and related biomarkers. In-depth phenotype information coupled with a complete picture of common and rare genetic variation available from the sequencing study provides an opportunity to use genetics as an instrument to better understand the role of MAPK-α/β in common diseases (including acute coronary syndrome) and its relationship to related biomarkers. It is anticipated that the low-frequency range (0.1% to 5% minor allele frequency [MAF]) could contain functional variation with larger effect sizes than that observed with common variation (>5% MAF).[5] Should such variants be found that mimic on-target effects, they may make useful tools for predicting drug effects and suggesting alternative indications for MAPK-α/β modulators.

Variants identified by Nelson et al and present in the Genetic Epidemiology of Metabolic Syndrome Study (GEMS)[6] and Cohorte Lausannoise (CoLaus) study[7] were profiled for association with cardiovascular and metabolic phenotypes and related biomarkers (38 and 40 traits, respectively). Analyses of these traits were performed on all sequenced subjects for GEMS (n=1576) and CoLaus (n=2086) and within dyslipidemic subjects for GEMS (n=787). The variants identified as associated in these initial analyses were evaluated in a small replication study (only myeloperoxidase [MPO] and glomerular filtration rate [GFR] results were obtained) within the Cardiovascular Health Study (CHS) and Framingham Heart Study (FHS). The same variants were then analyzed more broadly for association with 40 cardiovascular and metabolic traits in a meta-analysis in an expanded set including CoLaus, Life Sciences Prospective Population Study (LOLIPOP), European Prospective Investigation of Cancer (EPIC)-Norfolk, Ely, Fenland, and Genetics of Obesity Associations (GenOA) studies (Table 1 provides a summary of samples analyzed and Figure 1 a study flow diagram) to provide a cardiovascular and metabolic profile. We summarize the results and describe how they may relate to clinical trial results.

Materials and Methods

Population Characteristics

GEMS Study

The GEMS study is a large multinational study designed to explore the genetic basis of the metabolic syndrome.[6] Subjects were recruited from 2 centers in Europe (Oulu, Finland and Lausanne, Switzerland), 1 in the United States (Dallas, TX), 1 in Canada (Ottawa, Ontario), and 1 in Australia (Adelaide, South Australia). Dyslipidemic subjects were required to have the combination of an elevated plasma triglyceride (>75th percentile) and a low serum high-density lipoprotein (HDL)-cholesterol (<25th percentile) for their age, sex, and country threshold (age 18 to 75 years) and were nondiabetic. Unrelated normolipidemic controls were required to have plasma triglyceride lower than 50th percentile, serum HDL cholesterol >50th percentile for their age, sex, and country threshold, body mass index (BMI) >25 kg/m^2, and be >40 years of age. The subjects have phenotypes for cardiovascular and metabolic traits as well as biomarkers of inflammation. Dyslipidemic subjects (n=787 subjects) and normolipidemic controls (n=792 subjects), matched by sex, age, and collection center were sequenced. In some analyses, only dyslipidemic subjects were analyzed.

Cardiovascular Health Study

The CHS is a population-based, observational study of risk factors for clinical and subclinical cardiovascular diseases.[8] The study recruited participants 65 years and older from 4 US communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania) in 2 phases: 5201 participants from 1989 to 1990, and 687 (primarily African American participants) from 1992 to 1993. CHS participants completed standardized clinical examinations and questionnaires at study baseline and at 9 annual follow-up visits. Follow-up for clinical events occurs every 6 months. MPO was measured from frozen plasma samples (n=3044) collected at the 1992–1993 CHS examination.

Table 1. Summary of the Sample Characteristics and Phenotypes Analyses for Each Sample

	GEMS Dyslipidemia		GEMS Normolipidemia		CHS		FHS		GenOA Obesity Cases		GenOA Obesity Controls	
N	787		792		3897		2819		860		947	
Categorical variable, N (%)												
Men	467 (59%)		459 (58%)		1707 (44%)		1308 (46%)		241 (28%)		376 (40%)	
Statins	339 (43%)		21 (3%)		76 (2%)							
Lipid med	398 (51%)		21 (3%)		203 (5%)		584 (21%)					
Disease status, N (%)												
CAD	188 (24%)		18 (2%)		744 (19%)							
Dyslipidemia	787 (100%)		0 (0%)									
Obesity	284 (61%)		184 (39%)		1083 (28%)		852 (30%)		860 (100%)		947 (0%)	
T2D	0 (0%)		0 (0%)		1104 (28%)		366 (13%)					
Continuous variable (N, Mean (SD))												
Age, y	775	49 (9)	781	55.5 (9.3)	3897	72.8 (5.6)	2819	61 (9)	860	49.9 (10.7)	947	44.7 (15.1)
Hypertension												
DBP, mm Hg	771	82 (11)	780	81 (11)	3886	70 (11)	2819	74 (10)	857	82 (10)	947	74 (9)
SBP, mm Hg	771	132 (17)	780	132 (18)	3890	135 (21)	2819	127 (19)	858	135 (18)	947	121 (15)
Inflammation												
Adiponectin, μg/mL	743	5.5 (3.9)	757	8.1 (5.2)			2352	10 (6.3)				
CRPU, mg/L	743	6 (10)	757	5 (8)								
Fibrinogen, g/L	605	2.6 (0.62)	689	2.55 (0.64)	3874	3.2 (0.65)	2808	3.8 (0.74)				
IL-1b, pg/mL	603	47.9 (115)	689	47.5 (103)								
IL-6, pg/mL	603	45 (72)	689	51 (64)			2810	4 (4.8)				
IL-8, pg/mL	603	38.2 (76.6)	689	48.4 (80.3)								
Leptin, ng/mL	685	16.1 (11.7)	696	14.5 (12.2)								
MPO, ng/mL	605	52 (104)	690	43 (51.7)	3044	49 (49)	2722	48 (31)				
TNFA pg/mL	717	88 (132)	821	112 (425)			2144	145 (120)				
Insulin sensitivity												
2-h glucose, mmol/L							958	7.2 (2.7)				
Glucose, mmol/L	683	5.2 (0.6)	752	5.1 (0.5)	3897	6.1 (1.8)	2771	5.8 (1.4)	858	5.8 (2.0)	945	4.7 (0.5)
HOMA-B							2730	142 (376)				
HOMA-IR	651	3.1 (2.5)	728	1.8 (1.3)			2730	4.11 (3.65)	847	2.98 (2.7)	941	0.78 (0.41)
Insulin, mIU/mL	743	12.9 (9.6)	753	7.7 (5.8)	3866	16.6 (23)	2730	15.24 (10.6)	848	12.4 (9.7)	941	4.38 (2.3)
Kidney function												
Creatinine, μmol/L					3897	84 (26.7)						
CRU, mmol/day												
GFR							2819	85 (18.9)				
MACR												
MALB												
Lipid												
APOB, g/L	743	1.2 (0.3)	756	1.04 (0.24)								
CHOL, mmol/L	775	5.7 (1.2)	781	5.5 (0.9)	3895	212 (39)	2819	5.2 (0.9)	859	5.3 (1.02)	945	5.1 (0.97)
HDL, mmol/L	775	0.9 (0.2)	781	1.6 (0.3)	3890	1.4 (0.4)	2818	1.4 (0.4)	854	1.27 (0.36)	945	1.63 (0.42)

Continued

Table 1. Continued

	GEMS Dyslipidemia		GEMS Normolipidemia		CHS		FHS		GenOA Obesity Cases		GenOA Obesity Controls	
LDL, mmol/L	618	3.4 (1.1)	781	3.4 (0.9)	3836	3.37 (0.9)	3778	3.1 (0.84)	837	3.24 (0.89)	943	3.07 (0.83)
TRIG, mmol/L	775	3.5 (2.2)	781	1 (0.3)	3895	1.6 (0.85)	2819	1.6 (1.0)	858	1.76 (1.0)	945	0.94 (0.5)
Liver function												
ALB, g/L												
ALP, U/L												
ALT, U/L												
GGT, U/L												
Obesity												
BMI, kg/m^2	775	28.7 (3.6)	780	28.3 (3.7)	3897	26.4 (4.5)	2819	28.2 (5.3)	857	40.7 (8.96)	947	20.68 (2.03)
Body fat, %												
Hip, cm	764	108 (8.1)	778	108.3 (8.9)	3885	102 (9.6)	2785	105 (10.4)				
Waist, cm	765	98.3 (10.6)	778	95.8 (12.4)	3897	94 (12.8)	2789	100 (14.1)	856	116 (20.3)	946	78.6 (7.5)
Weight, kg	775	84.7 (13.6)	781	83.2 (14)	3897	159 (31.5)	2818	79.5 (17.5)	858	114 (29)	947	61.1 (10.2)
Waist/hip ratio					3879	0.92 (0.1)	2782	0.95 (0.08)				
Others												
Calcium, mmol/L												
Uric acid, μmol/L					3897	339 (89)						

	CoLaus		LOLIPOPW		EPIC		Fenland		ELY	
N	5846		6565		20 370		6379		1722	
Categorical variable, N (%)										
Men	2778 (48%)		4264 (65%)		9604 (47%)		2938 (46%)		792 (46%)	
Statins	498 (9%)		1106 (17%)							
Lipid med					308 (2%)		173 (3%)		133 (8%)	
Disease status, N (%)										
CAD					1369 (7%)					
Dyslipidemia	520 (9%)		729 (11%)		2258 (11%)		748 (12%)		208 (12%)	
Obesity	904 (15%)		1736 (26%)		2974 (15%)		1238 (19%)		380 (22%)	
T2D	385 (7%)		511 (8%)		644 (3%)		81 (1%)		93 (5%)	
Continuous variable (N, Mean (SD))										
Age, y	5846	53 (10.7)	6565	52.9 (11.4)	20,364	59.3 (9.2)	6378	46.7 (7.3)	1722	61.1 (9.1)
Hypertension										
DBP, mm Hg	5845	79.3 (11.0)	6563	79.6 (10.6)	17 532	82.3 (11.0)	6373	75.6 (10.1)	1714	78.7 (10.3)
SBP, mm Hg	5845	128 (18.1)	6563	131 (19.4)	17 532	135 (18.1)	6374	123 (15.3)	1714	132 (16.5)
Inflammation										
Adiponectin, μg/mL	5773	10 (8)					4944	6.5 (3.4)	763	7.5 (3.7)
CRPU, mg/L	5636	2.5 (3.5)	6236	4.2 (7.9)	13 616	2.9 (4.3)	4847	2.9 (4.5)	655	1.9 (3.4)
Fibrinogen, g/L					16 169	2.9 (0.8)				
IL-1b, pg/mL	3599	6.3 (31)								
IL-6, pg/mL	5340	10 (107)							395	1.9 (3.2)
IL-8, pg/mL					1152	36 (201)				
Leptin, ng/mL	3558	13.6 (10.8)					4941	15.8 (16.6)	1708	22.1 (22.8)

Continued

Table 1. Continued

	CoLaus		LOLIPOPW		EPIC		Fenland		ELY	
MPO, ng/mL					2534	102 (79)				
TNFA, pg/mL	5730	6.4 (54.3)								
Insulin sensitivity										
2-h glucose, mmol/L	510	5.9 (2.6)					6201	5.3 (1.6)	1589	6.4 (2.4)
Glucose, mmol/L	5636	5.5 (1.1)	6565	5.4 (1.7)			6319	4.8 (0.5)	1717	5.1 (0.8)
HOM-AB	5000	92 (72)	6257	119 (157)			6235	124 (84)	1707	136 (90)
HOMA-IR	5000	2.3 (2.3)	6257	2.7 (3.8)			6238	1.72 (1.4)	1707	2.25 (1.63)
Insulin, mIU/mL	5171	8.8 (6.2)	6257	10.2 (10.3)			6261	6.7 (4.7)	1709	8.4 (5.2)
Kidney function										
Creatinine, μmol/L	5635	80 (22.2)	6564	90.6 (18.6)	13 654	86.3 (18.2)	6274	76.2 (14.7)	792	84.5 (15.9)
CRU, mmol/day	5508	13.3 (6.7)					6360	10.5 (7.7)		
GFR	5635	78.7 (15.7)	6562	92.4 (27)	13 654	75.8 (21.4)				
MACR	5507	1.6 (7.0)					4637	1.3 (3.4)		
MALB	5507	18.9 (80.1)					4637	12.9 (23)		
Lipid										
APOB, g/L	5788	1.74 (1.34)			13 469	1 (0.2)	4942	1 (0.2)		
CHOL, mmol/L	5636	5.6 (1)	6565	5.4 (1.1)	17 005	6.2 (1.2)	6331	5.4 (1)	1719	5.6 (1.1)
HDL, mmol/L	5636	1.6 (0.4)	6565	1.4 (0.4)	16 320	1.4 (0.4)	6331	1.5 (0.4)	1717	1.5 (0.4)
LDL, mmol/L	5554	3.3 (0.9)	6421	3.3 (0.9)	16 321	4.0 (1.0)	6281	3.4 (0.9)	1715	3.5 (0.9)
TRIG, mmol/L	5636	1.4 (1.1)	6564	1.5 (1.2)	17 003	1.93 (1.1)	6331	1.2 (0.8)	1717	1.4 (0.8)
Liver function										
ALB, g/L	5636	44.2 (2.5)	6565	43.5 (2.8)	13 780	41.5 (5.7)	6330	42.1 (2.6)	497	43.3 (2.9)
ALP, U/L	5636	63.6 (20.5)	6558	77.4 (31.1)	13 826	60.3 (20.3)	6324	80.8 (21.3)		
ALT, U/L	5636	27.8 (19.6)	6563	28.7 (19.6)			6328	28.7 (15.9)		
GGT, U/L	5636	33.3 (59.5)	6565	43.2 (68.1)	13 869	32.8 (28.6)	6329	34.2 (25.5)		
Obesity										
BMI, kg/m^2	5844	25.8 (4.6)	6563	27.6 (5.1)	17 540	26.1 (3.9)	5836	26.9 (4.8)	1719	27.2 (4.7)
Body fat, %	5792	29.4 (9.2)	6290	30.5 (8.9)	11 534	31.9 (11.1)	5761	30.3 (8.8)	1698	33.7 (8.9)
Hip, cm	5842	101.7 (9.3)	6550	103.3 (9.5)			5832	103.6 (9.2)	1707	106.1 (10)
Waist, cm	5845	89.3 (13.4)	6550	95.5 (13.8)	17 553	88.0 (12.4)	5835	91 (13.3)	1709	93 (13.2)
Weight, kg	5845	73.6 (15.2)	6563	80.7 (16.9)			5836	78.1 (16.3)	1720	76.4 (15)
Waist hip ratio	5842	0.9 (0.1)	6549	0.9 (0.1)	17 536	0.86 (0.1)			1707	0.88 (0.1)
Others										
Calcium, mmol/L	5636	2.2 (0.1)	6564	2.3 (0.1)			6324	2.2 (0.1)	792	2.1 (0.1)
Uric acid, μmol/L	5636	314 (85)	6556	314 (89)	13 708	295 (81)				

ALB indicates albumin; ALT, alanine aminotransferase; APOB, apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; CHOL, cholesterol; CHS, Cardiovascular Health Study; CoLaus, Cohorte Lausannoise; CRPU, ultrasensitive C-reactive protein; CRU, urinary creatinine; DBP, diastolic blood pressure; EPIC, European Prospective Investigation of Cancer; FHS, Framingham Heart Study; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; GenOA, Genetics of Obesity Associations; GFR, glomerular filtration rate; GGT, γ-glutamyl transferase; glomerular filtration rate; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment – beta-cell; HOMA-IR, homeostatic model assessments – insulin resistance; IL, interleukin; LDL, low-density lipoprotein; LOLIPOPW, London Life Sciences Prospective Population Study – Whites; MACR, microalbumin-creatinine ratio; MALB, microalbuminuria; MPO, myeloperoxidase; SBP, systolic blood pressure; T2D, type 2 diabetes; TNFA, tumor necrosis factor α; TRIG, triglycerides.

Framingham Heart Study

The FHS was initiated in 1948 and recruited, from the town of Framingham, MA, a total of 5209 participants, most from European ancestry, who have undergone biannual examinations to study cardiovascular disease and related risk factors.[9] The Offspring cohort was recruited in 1971 and

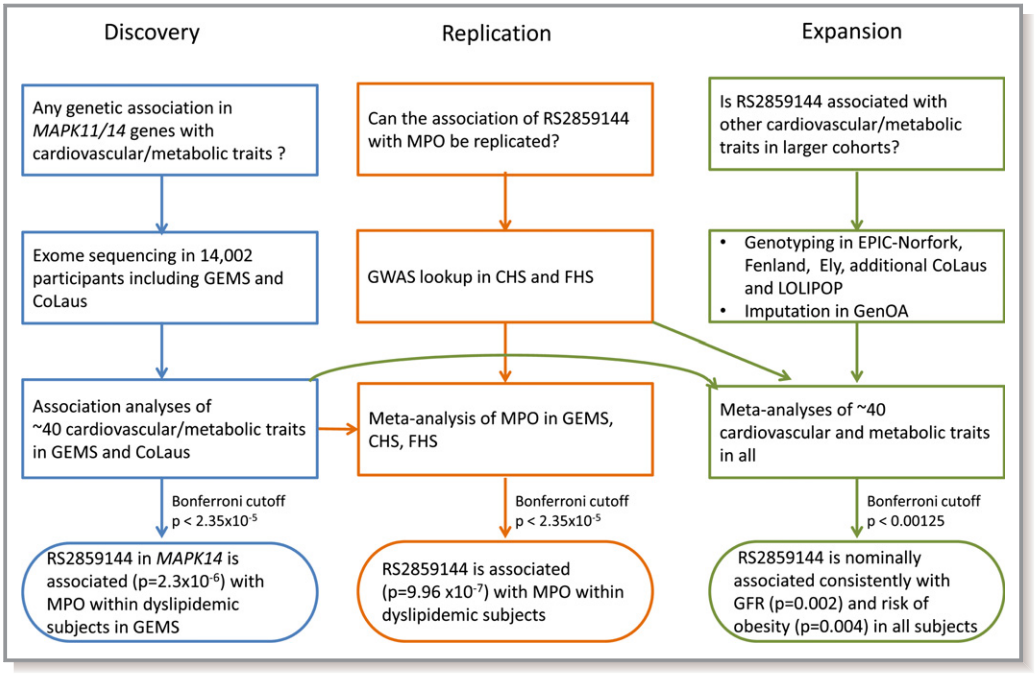

Figure 1. Study flow chart. CHS indicates Cardiovascular Health Study; CoLaus, Cohorte Lausannoise; FHS, Framingham Heart Study; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; GenOA, Genetic Obesity Associations; GFR, glomerular filtration rate; GWAS, genome-wide association study; LOLIPOP, London Life Sciences Prospective Population Study; MPO, myeloperoxidase.

includes 5124 children of the original cohort and their spouses.[10] Serum MPO measures was available for 2940 FHS Offspring cohort participants during the seventh cycle of examination (1998–2001).

CoLaus Study

The CoLaus study is a community-based study of 6188 European white subjects aged 35 to 75 years. Participants were drawn from the CHUV University Hospital in Lausanne Switzerland[7] and studied for cardiovascular and metabolic phenotypes.

LOLIPOP Study

The London Life Sciences Prospective Population Study (LOLIPOP) is a population-based study of 21 915 subjects identified from the lists of 58 general practitioners in West London.[11] Participants are primarily Indian Asians and European whites aged 35 to 75 years, who have been characterized for cardiovascular phenotypes. Only European whites (n=6565) were included in this analysis.

EPIC-Norfolk

The EPIC-Norfolk study is a cohort study investigating the relationship between diet and incident disease.[12] Over 25 639 men and women aged between 45 and 74 were recruited in Norwich and the surrounding area. Subjects were characterized for cardiovascular and metabolic phenotypes. MPO was available in nonfasted baseline serum samples for 1138 incident coronary artery disease cases and 2237 controls after an 8year follow-up.

Fenland

The Fenland Study is an ongoing, population-based cohort study (started in 2005) designed to investigate the association between genetic and lifestyle environmental factors and the risk of obesity, insulin sensitivity, hyperglycemia, and related metabolic traits in men and women aged 30 to 55 years.[13] Participants were recruited from General Practice sampling frames in the Fenland, Ely, and Cambridge areas of the Cambridgeshire Primary Care Trust in the United Kingdom. After an overnight fast, participants underwent a detailed clinical examination, and blood samples were collected. Data from up to 5105 participants were included in the current analyses.

Ely

The MRC Ely Study is a population-based cohort randomly selected from people living in Ely and surrounding villages (East Anglia, United Kingdom), an ethnically homogeneous European ancestry population. The study design, methods, and measurements of the 3 phases have been described in detail elsewhere.[14] The current analyses included

individuals aged 35 to 79 years, from phase 3. Data from up to 1606 participants were included in the current analyses.

GenOA

The Genetics of Obesity Associations (GenOA) study is a case–control study with 1008 obese (BMI >30) white subjects recruited from the Ottowa obesity weight management clinic and 991 white controls (BMI <40th percentile for age and gender) from the local community.[15] Obesity and type 2 diabetes status were meta-analyzed with other studies in overall subjects.

Ethical oversight for genetic research and patient informed consent were obtained by each specific study.

Sequencing

The MAPK11 and MAPK14 genes were sequenced along with 200 other genes in 14 002 subjects.[4]

MPO Assay

MPO was measured in fasting serum for GEMS, FHS, and CHS and nonfasting serum for EPIC-Norfolk. MPO was determined in GEMS by Pathway Diagnostics (Cypress, CA) using the Myeloperoxidase ELISA kit #K6631 from ALPCO Diagnostics (Salem, NH). CHS and EPIC-Norfolk both used the CardioMPO test (PrognostiX Inc, Cleveland, OH), a Food and Drug Administration–approved sandwich enzyme–linked immunosorbent assay. Normal control values from a middle-aged healthy population have been reported to be <640 pmol/L. The minimum detection limit (calculated using interpolation of the mean plus 2 SDs) was 30 pmol/L, and a within-run precision of 4.8% was reported.[16] In the FHS, a quantitative ELISA kit was used (Oxis, Cat. No. 21013) and read on a Molecular Devices VersaMax microplate reader. The minimum detectable dose was 0.17 ng/mL, the standard curve range was 0 to 25 ng/mL, and the intra-assay variability was 3.15%.

Phenotypes Analyzed

Table 1 characterizes each sample set analyzed and lists all phenotypes analyzed in the initial analyses of the GEMS collection, replication analyses for the CHS and FHS, and profiling analysis for the CoLaus, LOLIPOP, EPIC-Norfolk, Fenland Ely, and GenOA studies. Table 2 characterizes the dyslipidemic subgroups only.

Obese participants were required to have a BMI ≥30 kg/m^2 and age ≥18 years. Lean controls were required to have a current BMI that is ≤40th percentile for their age and sex groups and not previously reported having had a BMI >25th percentile for age and sex for more than a 2 -year consecutive period and age ≥18 years. Dyslipidemic subjects were selected for high triglycerides (≥75th percentile) and low HDL cholesterol (≤25th percentile) based on age, sex, and country threshold. Normolipidemic subjects were selected for high HDL cholesterol (above median), low triglycerides (below median), and BMI >25.[6]

Genotyping

CHS

Genotyping for RS612049 was performed using the custom IBCv2 genotyping array that contains high single nucleotide polymorphism (SNP) marker density and LD coverage for ≈2100 genes related to cardiovascular, inflammation, hemostasis/coagulation, and metabolic phenotypes.[17]

FHS

RS612049, a genome wide association study marker, from the Affymetrix 500K array was available and observed to be in perfect linkage disequilibrium with RS2859144 in the Hapmap CEU samples.

CoLaus and LOLIPOP

RS2859144 was genotyped in the entire CoLaus collection and 6565 European white subjects from the LOLIPOP collection at KBiosciences using KASPar technologies (Hoddeson, UK).

EPIC, Ely, Fenland, and EPIC-Norfolk

RS612049 was genotyped at MRC Cambridge labs using Sequenom technology (2 plexes).

GenOA

RS2859144 was assessed in this collection by imputation. Reference haplotypes (NCBI Build 36) from the sequencing study (n=3983) were imputed into cases (810) and controls (830) from the GenOA collection with Affymetrix 6.0 array data using BEAGLE[18] with the default settings. RS2859144 was well imputed with an imputation r^2=0.898.

Statistical Methods

Initial analysis of the variants identified by sequencing for the *MAPK14* and *MAPK11* genes for the GEMS and the subset of CoLaus samples was carried out using an additive genetic model with linear or logistic regression and up to 40 cardiovascular and metabolic traits. A natural log-transformation was applied for quantitative traits with non-normal distributions after outliers were winsorized at 99.9th percentile of the distribution. Quantitative trait analyses were based

DOI: 10.1161/JAHA.114.001074

Table 2. Population Information in Dyslipidemia Subset

	GEMS		CoLaus		LOLIPOPW		CHS		FHS		EPIC		Fenland		ELY	
N	787		520		729		433		391		2258		748		208	
Categorical variable, N (%)																
Men	467 (59%)		276 (53%)		457 (63%)		266 (61%)		166 (42%)		1018 (45%)		328 (44%)		98 (47%)	
Statins	339 (43%)		70 (14%)		153 (21%)		12 (3%)				44 (2%)		34 (5%)		21 (10%)	
Continuous variable (N, Mean (SD))																
Age, y	775	49.2 (9.1)	520	54.3 (10.1)	729	52.7 (11.3)	433	72.3 (5.3)	391	61.6 (9.5)	2258	59.4 (9.2)	748	46.9 (7.2)	208	61.5 (8.3)
Hypertension																
DBP, mm Hg	771	81.7 (10.9)	520	81.5 (11.5)	729	81.4 (9.9)	433	70.2 (10.9)	391	75.2 (10.2)	2256	84.7 (10.5)	747	79.8 (9.8)	208	82.1 (10.4)
SBP, mm Hg	771	131.9 (17.3)	520	131.9 (18.4)	729	133.2 (19)	433	136 (21.7)	391	130.9 (18.1)	2256	138.7 (17.6)	747	127.3 (15.1)	208	136.1 (17.4)
Inflammation																
Adiponectin, μg/mL	743	5.5 (3.9)	518	6.64 (4.57)					329	6.58 (3.96)			605	4.6 (2.2)	95	6.0 (3.0)
CRPU, mg/L	743	6 (10)	520	3.7 (4.2)	695	5.8 (6.6)					1769	3.4 (3.9)	605	4.1 (4.4)	80	2.1 (2.3)
Fibrinogen, g/L	605	2.6 (0.62)					431	3.27 (0.64)	390	3.99 (0.76)	2103	3.0 (0.8)				
IL-1b, pg/mL	603	47.9 (115)	320	6.7 (20.3)												
IL-6, pg/mL	603	45 (72)	496	18.4 (209.4)					390	4.45 (4.43)					43	1.5 (1.2)
IL-8, pg/mL	603	38.2 (76.6)									170	60.4 (281.3)				
Leptin, ng/mL	685	16.1 (11.7)	345	17.9 (12.3)									605	25.4 (21.3)	206	30.1 (26.7)
MPO, ng/mL	605	52 (104)					311	49 (41)	391	45.8 (31.7)	352	102 (80)				
TNFA, pg/mL	717	88 (132)	512	5.7 (9.9)					305	145.5 (120.5)						
Insulin sensitivity																
2h glucose, mmol/L			45	6.9 (3.8)					138	8.7 (3.5)			728	6.2 (1.9)	190	7.9 (2.9)
Glucose, mmol/L	683	5.2 (0.6)	520	6.1 (1.9)	729	6.2 (2.6)	433	130 (52.2)	384	6.7 (2.4)			746	5.1 (0.6)	208	5.4 (1)
HOMA-B			497	114.8 (88.2)	700	175.2 (155.7)			378	169.5 (128.1)			738	166.8 (99.5)	206	164.7 (88.9)
HOMA-IR	651	3.1 (2.5)	497	3.8 (4.7)	700	5.3 (8)			378	6.96 (6.44)			738	2.9 (2)	206	3.5 (2.1)
Insulin, mIU/mL	743	12.9 (9.6)	497	13.1 (8.8)	700	17.9 (17.9)	428	27.9 (42.4)	378	22.19 (16.8)			740	10.7 (6.4)	206	12.1 (6.2)
Kidney function																
Creatinine, μmol/L			520	81.9 (22)	729	92.4 (27.3)	433	1.18 (0.40)			1770	84.4 (19.3)	743	77.6 (15.2)	97	88.0 (17.0)

Continued

DOI: 10.1161/JAHA.114.001074

Table 2. Continued

	GEMS		CoLaus		LOLIPOPW		CHS		FHS		EPIC		Fenland		ELY	
CRU, mmol/day			504	14 108 (7139)									743	11.9 (8.1)		
GFR			520	77.8 (16)	729	103.1 (32.1)			391	80.6 (20.8)	1770	77.8 (22.9)	743	87.2 (16.4)		
MACR			504	2231 (6959)									603	1.4 (2.9)		
MALB			504	27.5 (72.1)									603	16.4 (30.3)		
Lipid																
APOB, g/L	743	1.2 (0.3)	515	1.97 (1.63)							1752	1.05 (0.3)	605	1.1 (0.2)		
CHOL, mmol/L	775	5.7 (1.2)	520	5.8 (1.1)	729	5.5 (1.1)	433	206.0 (37.8)	391	5.26 (1.04)	2258	6.5 (1.1)	748	5.7 (1)		
HDL, mmol/L	775	0.9 (0.2)	520	1.1 (0.2)	729	1 (0.1)	433	35.0 (4.6)	391	0.9 (0.2)	2258	1.0 (0.2)	748	1.1 (0.2)	208	1.0 (0.2)
LDL, mmol/L	618	3.4 (1.1)	479	3.6 (1.0)	639	3.2 (0.9)	392	3.2 (0.98)	355	3.0 (0.97)	2258	4.2 (1.1)	709	3.6 (0.9)	206	3.7 (1)
TRIG, mmol/L	775	3.5 (2.2)	520	2.8 (2.1)	729	3.1 (1.9)	433	3.03 (1.44)	391	3.06 (1.45)	2258	3.0 (0.8)	748	2.3 (1)	208	2.8 (1.0)
Liver function																
ALB, g/L			520	43.8 (2.6)	729	43.2 (2.7)					1784	41.8 (5.8)	748	41.6 (2.7)	97	42.2 (3.3)
ALP, U/L			520	69.2 (21.1)	727	84.8 (37.4)					1789	64.6 (20.6)	747	85.2 (22.1)		
ALT, U/L			520	34 (21.6)	729	34.3 (25.2)							748	34.9 (19.4)		
GGT, U/L			520	39.1 (46.4)	729	56.6 (116.6)					1793	38.1 (30.9)	748	41.3 (28.8)		
Obesity																
BMI, kg/m^2	775	28.7 (3.6)	520	29.1 (4.7)	729	31 (5.6)	433	28.2 (4.4)	391	30.5 (5.3)	2254	28.2 (3.9)	687	30.6 (5.4)	208	29.7 (4.6)
Body fat, %			515	32.3 (9.7)	690	35.6 (8.8)					1412	35.8 (11.8)	677	35.1 (9)	205	36.1 (8.9)
Hip, cm	764	108 (8.1)	520	106.6 (9.7)	728	108.2 (10)	430	104 (9.2)	384	108 (10.8)			686	109.7 (10.6)	205	109.8 (10)
Waist, cm	765	98.3 (10.6)	520	98.2 (12.5)	728	104.5 (13.3)	430	100.3 (11.3)	384	105.9 (12.5)	2257	93.8 (11.5)	687	100.6 (12.9)	206	99.5 (11.4)
Weight, kg	775	84.7 (13.6)	520	82.5 (15.5)	729	90.4 (18)	433	174 (30.4)	391	84.7 (17.3)			687	88.6 (17.8)	208	82.3 (13.8)
Waist/hip ratio			520	0.9 (0.1)	727	1 (0.1)	430	0.96 (0.07)	383	0.98 (0.068)	2252	0.89 (0.09)	686	0.92 (0.08)	205	0.91 (0.1)
Others																
Calcium, mmol/L			520	2.2 (0.1)	729	2.3 (0.1)							747	2.2 (0.1)	97	2.1 (0.1)
URIC, μmol/L			520	353.7 (91.5)	726	340.6 (86.4)	433	386.6 (95.2)			1773	314.5 (83.6)				

ALB indicates albumin ; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CHS, Cardiovascular Health Study; CHOL, cholesterol; CoLaus, ; CRPU, ultrasensitive C-reactive protein; CRU, urinary creatinine; APOB, apolipoprotein B; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; EPIC, European Prospective Investigation of Cancer; FHS, Framingham Heart Study; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; GFR, glomerular filtration rate; GGT, γ-glutamyl transferase; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment – beta-cell; HOMA-IR, homeostatic model assessment – insulin resistance; IL, interleukin; LDL, low-density lipoprotein; LOLIPOPW, London Life Sciences Prospective Population Study – Whites; MACR, microalbumin-creatinine ratio; MALB, microalbuminuria; MPO, myeloperoxidase; SBP, systolic blood pressure; TNFA, tumor necrosis factor α; TRIG, triglycerides; URIC, uric acid.

on normalized residuals after adjusting for significant covariates including dyslipidemia status, age, sex, collection site, waist and hip circumference for the GEMS study and age, sex, BMI (if applicable), smoking, physical activity, and alcohol use for the CoLaus study. Binary traits were analyzed with and without adjustment of relevant covariates. SNPs with at least 10 copies of the minor allele were analyzed individually, whereas putatively functional SNPs with minor allele frequency <0.5% were aggregated.[19] There were 7 (*MAPK11)* plus 15 (*MAPK14)* variants analyzed individually in GEMS, and 6 (*MAPK11)* plus 13 (*MAPK14)* variants in CoLaus. Three aggregate tests were carried out for each gene: (1) all rare variants that lead to a change in the amino acid sequence (nonsynonymous, nonsense, readthrough, and splice site variants), (2) all rare, amino acid–changing variants that are predicted to be functional damaging by SIFT or PolyPhen, and (3) variants in (2) plus additional rare variants at a highly conserved base position (phyloP ≥2). Analyses were conducted in all subjects and in GEMS dyslipidemic only subjects). A Bonferroni-corrected significance threshold adjusting for the number of tested variants in both genes, number of traits, and number of subgroups analyzed was set at $P=0.05/(38\times(22+6)\times2)=2.35\times10^{-5}$ for the GEMS and $P=0.05/(40\times(19+6))=5.0\times10^{-5}$ for the CoLaus study, respectively.

Genetic association analyses of the genotyped variant (rs2859144 or proxy) for the full set of CoLaus, LOLIPOP, EPIC-Norfolk, Ely, Fenland, CHS, and FHS studies were carried out in each study (after transformation as described previously) under an additive genetic model using linear or logistic regression, and linear mixed-effect or generalized estimating equation models in FHS to account for familial correlation. To accommodate the uncertainty of imputed genotypes, allelic dosages in the GenOA study were analyzed as a continuous variable in linear/logistic regression. Obese-only and dyslipidemic-only subgroup analyses were performed to enable replication of some context-specific study results. In the CoLaus study, 330 subjects were removed from the analysis due to genetic relatedness (PI_HAT>0.125) based on identity by descent (IBD) sharing estimated from PLINK using existing Affy500K data. Summary statistics from all studies were meta-analyzed using the inverse variance method.[20] The *P* value threshold for replication of the MPO association in CHS and FHS was set at $P=0.05$. For the expanded cardiovascular and metabolic profile, a Bonferroni-corrected significance threshold was used for the meta-analysis, adjusting for the traits evaluated ($P=0.05/40=0.00125$).

GEMS Inflammatory Markers

Methods used for measurement of plasma lipids and glucose in serum have been described previously.[6] All additional biomarkers were measured by Pathway Diagnostics (Cypress, CA) using either plasma or serum. Frozen serum and multiple aliquots of EDTA plasma specimens were received at Pathway Diagnostics on dry ice. The specimens were stored at −70°C until thawed for testing. Since it was not logistically possible to perform all of the many assays for a given subject on the same day, plasma aliquots were not pooled. Instead, individual aliquots of serum and plasma were thawed as necessary and tested as efficiently as possible; that is, as many individual assays as was practical were run with the thawed aliquots within 8 hours of being thawed. While minor heterogeneity between aliquots was possible, this method minimized any risk of specimen stability that may have occurred if the specimens were stored for long periods at 2 to 8°C or via multiple freeze/thaw cycles. Once thawed, plasma was removed for each assay and diluted (if necessary, depending on the assay) using an assay-specific buffer.

Adiponectin was measured using an ELISA assay (R&D Systems, Minneapolis, MN); CRP and insulin were measured using chemiluminescent assays (Diagnostic Products Corp, Los Angeles, CA); LDL particle-size was measured using a LipoPrint kit from Quantimetrix Corporation (Redondo Beach, CA); leptin was quantified using an ELISA (American Lab Products Company, Windham, NH) and apo B was measured using an immunoturbidometric assay (Polymedco). PAI-1 was evaluated using the PAI-1 (Plasminogen Activator Inhibitor, Type 1) ELISA kit #EL504 from Dakocytomation (Carpinteria, CA). MPO was determined using the Myeloperoxidase ELISA kit #K6631 from ALPCO Diagnostics (Salem, NH). MMP9 was measured using the MMP9 (Total) Quantikine ELISA kit #DMP900 from R&D Systems (Minneapolis, MN). Oxidized LDL was evaluated in serum using the Oxidized LDL (Ab) EIA kit #04-BI-20032 from ALPCO Diagnostics. Multiplex testing of interleukin (IL)-1a, IL-1b, IL-6, IL-8, IL-10, IL-18, monocyte chemotactic protein 1 (MCP1), soluble vascular cell adhesion molecule, soluble intracellular adhesion molecule, and tumor necrosis factor α were performed using customized kits purchased from Beckman Coulter (Fullerton, CA) for determination using the Beckman Coulter A-Square™ reader. Prothombin F1+2 and Fibrinogen were determined using the Prothombin F1+2 ELISA kit #REFOWVVII from Dade-Behring (Newark, DE) and the Fibrinogen (Clauss) kit #0008469110 by Instrument Laboratory (Milan, Italy), respectively. Instructions provided by the manufacturer were followed for each test kit. All colorimetric determinations were made using a Tecan GENios Pro™ (Grodig, Austria) plate reader and analyzed using Magellan™ software except for the multiplex tests, which were performed using the Beckman Coulter A-Square™ reader and Fibrinogen, which was evaluated on the ACL Advance™ (Beckman Coulter).

While citrated plasma was the preferred matrix for the determination of Prothrombin F1+2 and Fibrinogen, only EDTA

plasma was available for testing. A small comparison study performed at Pathway Diagnostics of EDTA plasma versus citrated plasma from healthy volunteers was performed and yielded similar results. At the request of GSK, Prothrombin F1+2 and Fibrinogen were performed on the EDTA specimens.

Results

The resequencing (exons, intron–exon boundaries, and untranslated regions) of the *MAPK14* and *MAPK11* genes (7844 bp total) in 14 002 ethnically diverse subjects including well-phenotyped subjects from the GEMS (n=1579) and CoLaus (n=2086) collections has been described previously.[4] Within all 14 002 subjects, we identified 232 and 157 SNPs, including 2822 and 27 amino acid changes and splice sites, as well as 135 and 78 SNPs in the untranslated region, respectively. For *MAPK14*, variants included a splice variant (RS200906391) common (MAF>0.5%) in all ethnicities and an additional coding variant present only in African Americans (S64Y). Additionally, there were 2 predicted functional (PHYLOP>2) low-frequency variants L250F (26 carriers) and D343G (35 carriers) found mainly in Europeans. The remaining predicted functional variants were very rare, mostly private mutations. For *MAPK11*, we observed 1 common nonsynonymous variant in all ethnicities (R275H) and only E177K was predicted to be functional (PHYLOP>2) and was not private in Europeans. Variants identified in the *MAPK11* and *MAPK14* genes were reported previously.[4] Figure 2 summarizes variation found in 12 514 European white, 594 African American, and 567 South Asian subjects for the *MAPK14* gene and Figure 3 for the *MAPK11* gene.

Common and rare variation in the *MAPK14* and *MAPK11* genes and flanking regions was analyzed for association with cardiovascular, metabolic, and inflammatory biomarker traits available in GEMS (n=1579) and the sequenced subset of CoLaus (n=2086). In GEMS, analyses for the *MAPK14* and *MAPK11* genes included 15 and 7 common (MAF>0.5%) variants individually, while aggregated analyses included 12 and 6 rare variants, respectively. In CoLaus, single-variant analyses for the *MAPK14* and *MAPK11* genes included 13 and 6 common SNPs, while aggregated analyses included 10

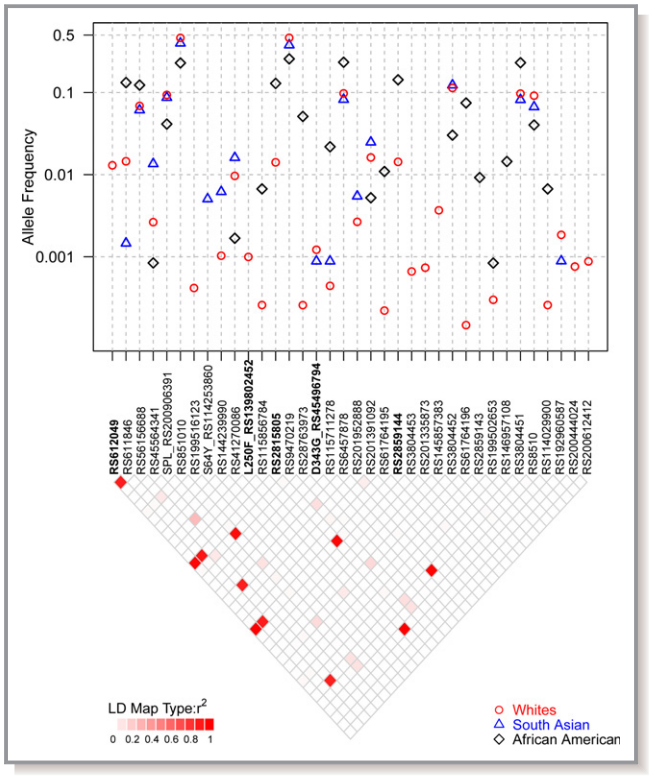

Figure 2. Sequence variants identified in European (whites), South Asian, and African American samples for *MAPK14* gene. The figure summarizes the MAF for all variants with more than 10 copies observed in subjects sequenced in Nelson et al[4] along with selected variants present in 1000 genomes and used in our analyses. Frequencies are shown by ethnic group. The 2 rare coding variants (D343G and L250F), 2 key SNPs (RS2859144 and RS2815805), and RS612049 from SLC6A8 in high LD are shown in bold. The LD for all variants in European subjects is shown as well. LD indicates linkage disequilibrium; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms.

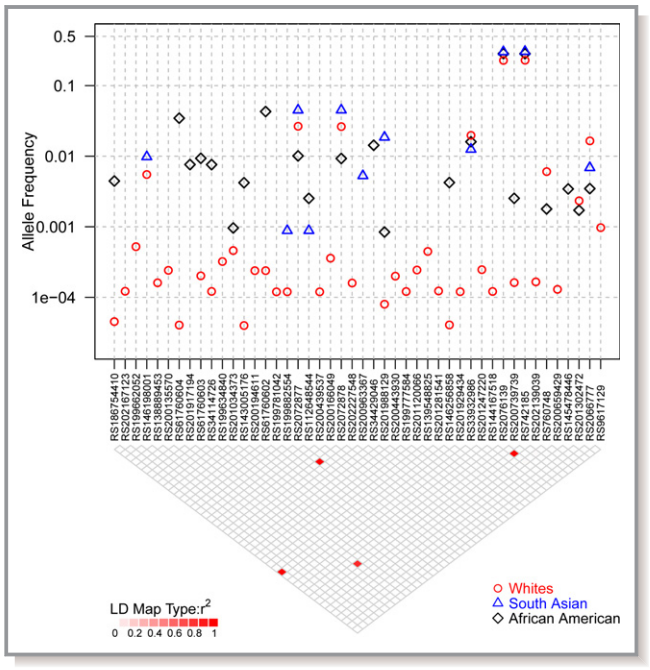

Figure 3. Sequence variants identified in European (whites), South Asian, and African American samples for the *MAPK11* gene. The figure summarizes the minor allele frequency for all variants with more than 2 copies observed in subjects sequenced in Nelson et al[4] Frequencies are shown by ethnic group. The LD for all variants in European subjects is shown as well. LD indicates linkage disequilibrium.

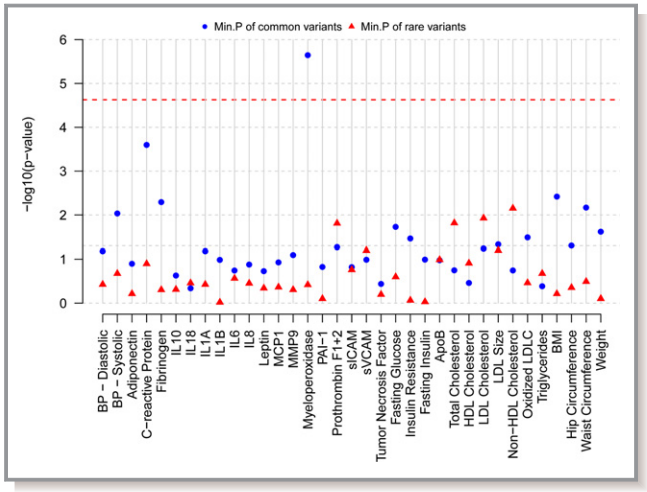

Figure 4. Analysis of cardiovascular, metabolic, and inflammatory biomarker phenotypes in GEMS collection for variants assayed via sequencing in the *MAPK14* gene. The figure summarizes the *P*-values for association tests performed for all available traits in the GEMS collection for the *MAPK14* gene within dyslipidemic subjects. For each trait, the minimum of the *P*-values for common variants and *P*-values for the 3 rare variant aggregated tests are plotted in this graph. Common variants are defined as those with MAF >0.5%. *P*-value thresholds are specified with a line and were calculated as 0.05/(no. of traits×no. of markers in both genes×no. of subgroups analyzed) $P=0.05/(38\times(22+6)\times2)=2.35\times10^{-5}$. ApoB, apolipoprotein B; BMI indicates body mass index; BP, blood pressure; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; LDLC, low-density lipoprotein cholesterol; MAF, minor allele frequency; PAI, plasminogen activator inhibitor; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule.

and 5 rare variants, respectively. Analyses were conducted in the GEMS collection on all samples (38 traits) and within the dyslipidemic subjects (35 traits) and in the CoLaus collection on all samples (40 traits). From this initial round of analysis, a significant association was noted for a low-frequency SNP, RS2859144 (1.5% MAF) in *MAPK14* with MPO within dyslipidemic subjects ($P=2.3\times10^{-6}$) in GEMS. This association is illustrated in Figure 4. This SNP was in tight linkage disequilibrium ($r^2=1$) with a synonymous SNP RS2815805 (H253H) and both had the same minor allele frequency. See Figure 5 for a gene schematic. Interestingly, RS2859144 was not associated with MPO in GEMS overall and on closer inspection, the allelic effect was observed to be opposite in normolipidemic controls as compared to the dyslipidemic cases (interaction $P=1.7\times10^{-6}$). Figure 6 summarizes the results for all traits within the GEMS (Figure 6A—dyslipidemic and normolipidemic combined) and CoLaus collections (B) for MAPK14, as well as MAPK11 (Figure 6C through 6E), including the minimum *P*-value for common SNPs and the *P*-value for aggregated analyses for rare variants. The thresholds for the analyses are highlighted in the graphs and specified in the figure legends. No other statistically significant associations (common or aggregated SNPs) were noted within the *MAPK14* and *MAPK11* genes; however, a nominally significant association ($P=0.016$) was observed between rare variants in *MAPK14* and IL-6 levels (Figure 7), a known pharmacodynamic marker of losmapimod.

As such interactions are often the result of chance, we sought replication in additional cohorts where MPO and dyslipidemia phenotypes were available. Neither RS2859144 nor RS2815805 were present on common genotyping platforms for CHS and FHS studies, but another marker in high linkage disequilibrium ($r^2=1$) was available (RS612049). Despite their low frequency, these variants are well-tagged by genome wide association study SNPs. We obtained genotype SNP data from CHS and FHS where RS612049, fasting lipids, and MPO were available in a population sample. Dyslipidemic and normolipidemic criteria from GEMS were applied to the 2 population-based cohorts, and analysis was performed in those subgroups. RS612049 was also genotyped in an EPIC-Norfolk sample where MPO levels were measured in nonfasting patients within a cardiovascular nested case–control study (cases n=1138 and controls n=2237).[21] As the incident cases were enriched for high MPO levels, given the relationship between coronary artery disease and MPO observed in this sample,[21] we included only

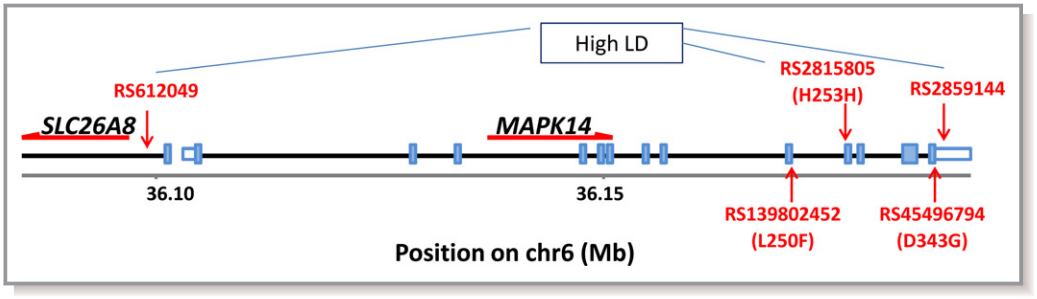

Figure 5. *MAPK14* gene region. The location of the 2 rare coding variants is shown below and the 3 low-frequency variants in high LD are shown above the gene schematic. LD indicates linkage disequilibrium.

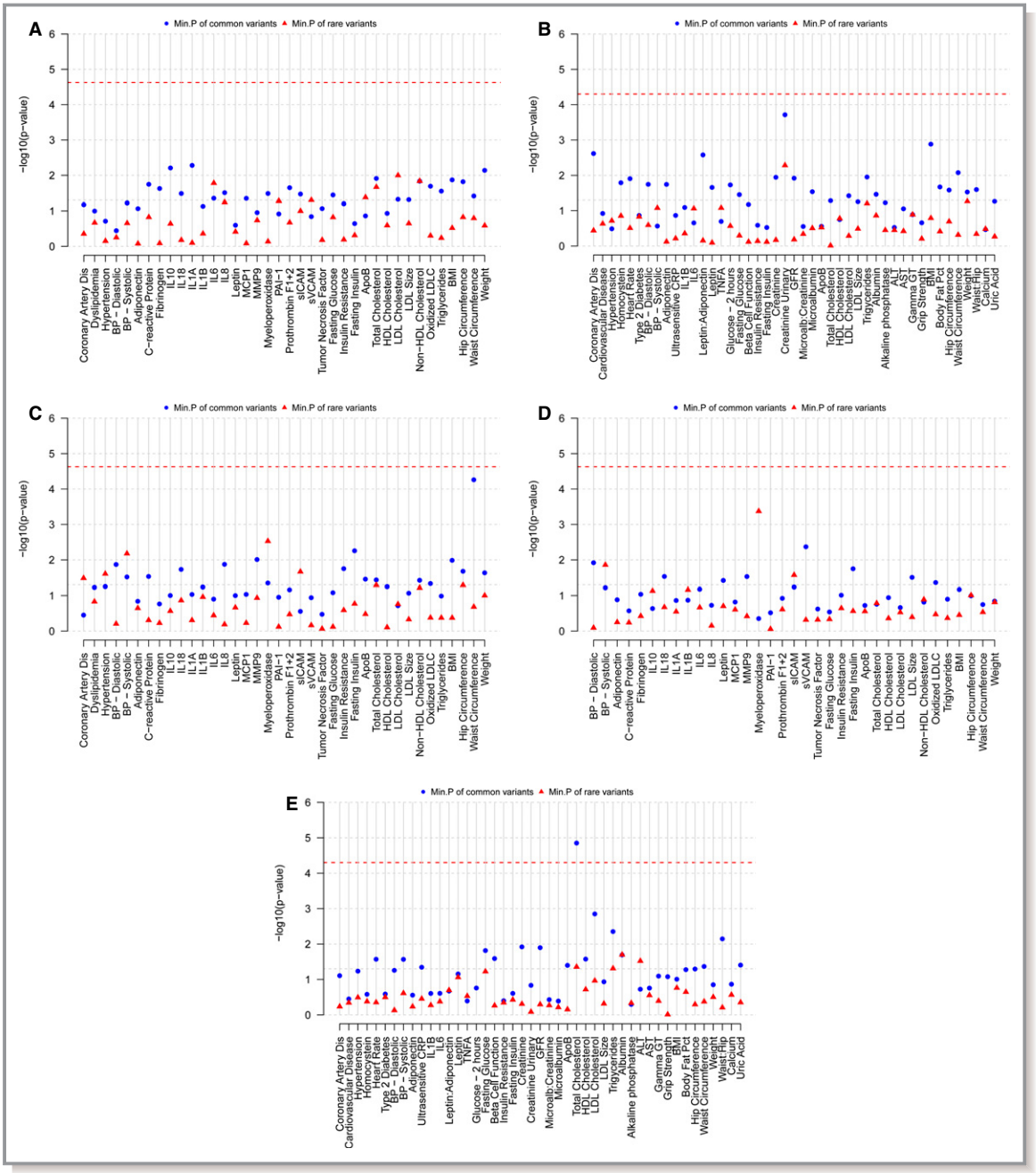

Figure 6. Analysis of cardiovascular, metabolic, and inflammatory biomarker phenotypes in GEMS and CoLaus collections for sequence variants in the *MAPK14* and *MAPK11* genes. The figure summarizes the *P*-values for association tests performed for all available traits in the GEMS and CoLaus collections for *MAPK14* and *MAPK11*. Analyses were performed in all GEMS subjects (A), and within 2086 CoLaus subjects (B) for *MAPK14* and in all GEMS subjects (C), within dyslipidemic GEMS subjects (D), and within 2086 CoLaus subjects (E). For each trait, the minimum of the *P*-values for common variants and *P*-values for the 3 rare variant aggregated tests are plotted in this graph. Common variants are defined as those with MAF >0.5%. *P*-value thresholds are specified with a line and were calculated as 0.05/(no. of traits×no. of markers in both genes×no. of subgroups analyzed) $P=0.05/(38\times(22+6)\times2)=2.35\times10^{-5}$ for the GEMS and $P=0.05/(40\times(19+6))=5.0\times10^{-5}$ for CoLaus studies, respectively. 0.05/(no. of traits× no. of markers in both genes×no. of subgroups analyzed) $P=0.05/(38\times(22+6)\times2)=2.35\times10^{-5}$ for the GEMS and $P=0.05/(40\times(19+6))=5.0\times10^{-5}$ for CoLaus studies, respectively. ALT, alanine aminotransferase; ApoB indicates apolipoprotein B; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CoLaus, Cohorte Lausannoise; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; GFR, glomerular filtration rate; gamma GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; LDLC, low-density lipoprotein cholesterol; MAF, minor allele frequency; PAI−1, plasminogen activator inhibitor−1; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule.

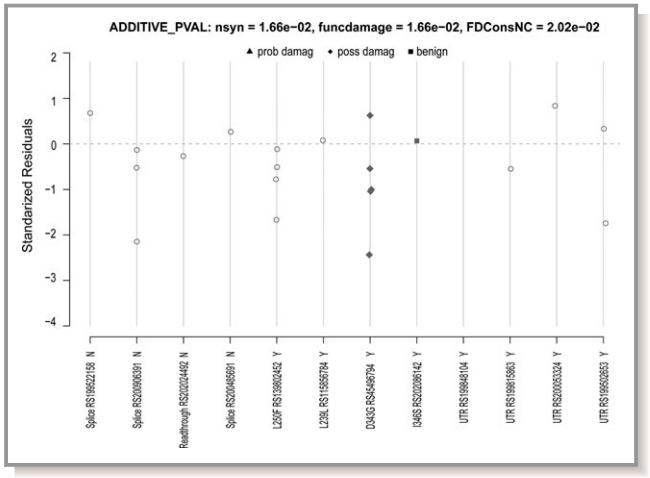

Figure 7. Rare variants in *MAPK14* and interleukin-6. Functional predictions are from SIFT.

the controls in the meta-analysis. As shown in Figure 8A, the CHS and FHS studies, but not the EPIC controls, provided additional evidence demonstrating an association with higher MPO levels in the dyslipidemic group (meta-P=7.56×10^{-6}) and no association within the normolipidemic subgroup (meta-P=0.62) in Figure 8B. The average effect size in the dyslipidemic group was quite substantial, with each additional

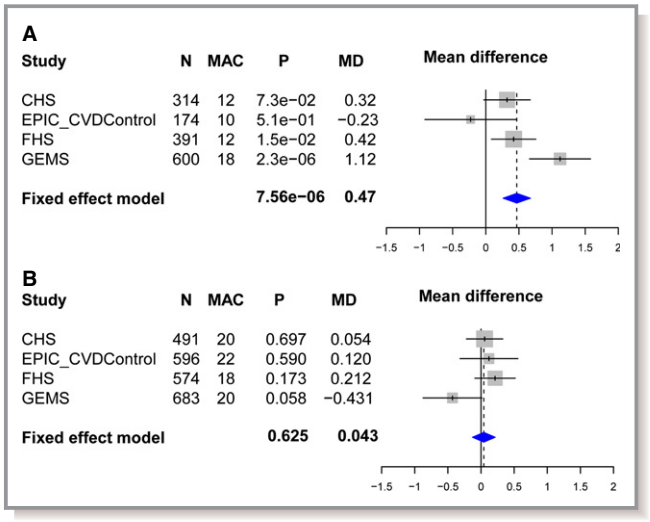

Figure 8. Association analyses for RS2859144/RS612049 and myeloperoxidase (MPO) levels by dyslipidemia status. The associations between *MAPK14* single nucleotide polymorphisms and MPO levels are shown by dyslipidemia (A) and normolipidemia (B) status in separate Forest plots (mean difference and 95% CIs). CHS indicates Cardiovascular Health Study; EPIC-CVD, European Prospective Investigation of Cancer–Cardiovascular Disease; FHS, Framingham Heart Study; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; MAC, minor allele count; MD, mean difference; P, p value.

copy of the minor allele corresponding to an average increase of 0.47 SD in MPO levels. The replication P value calculated by meta-analyzing FHS, CHS, and EPIC controls was significant, P=0.009 (0.31 SD average increase) for the dyslipidemic groups.

The variant associated with increased MPO levels was then analyzed for association with up to 40 cardiovascular and metabolic traits within the CoLaus, LOLIPOP, EPIC Norfolk, Ely, Fenland, and GenOA studies to provide further insight into the observed association. RS612049 was typed in Ely and Fenland, and RS2859144 was imputed in the GenOA case–control study and typed in LOLIPOP and remaining CoLaus samples to provide additional data. Although not significant after adjusting for the analysis of 40 traits (P=0.00125), there were some nominal associations that were directionally consistent (Figures 9 and 10). In the overall populations (Figure 10A), the same allele associated with higher MPO in dyslipidemic subjects appeared to confer a marginally lower GFR (Figure 9A, β −0.086, P=0.002) as well as an increased risk for obesity (Figure 9B, odds ratio 1.25, P=0.005). Nominal associations were also observed for higher uric acid (Figure 9C, P=0.01), though this was only available in a few studies. Within the dyslipidemic subgroups (Figures 9 and 10B), nominal associations were observed with lower systolic (Figure 9D, P=0.002) and diastolic blood pressures (Figure 9D, P=0.01) as well as lower HOMA-B (Figure 9E, P=0.03) and higher fasting glucose (Figure 9F, P=0.04). In addition, there were many more inflammation-linked markers available in GEMS than were available in the other studies and of those, RS2859144 was also nominally associated with oxidized-LDL in the dyslipidemic group (β −0.4, P=0.03).

Discussion

We sought to characterize gene variation in *MAPK11/14* genes in well-phenotyped individuals to identify genetic variants that can be used to predict drug effects and generate hypotheses around alternative indications for MAPK-α/β inhibitors including Losmapimod, a p38 map kinase inhibitor in development for acute coronary syndrome. Comprehensive sequencing meant that 94% of the variant alleles with minor allele frequency of 0.01% in Europeans were sampled at least once. The variants present in the GEMS and CoLaus collections were characterized using cardiovascular, metabolic, and biomarker phenotypes. We identified an association of RS2859144 in *MAPK14* with MPO in a dyslipidemic population (GEMS, P=2.3×10^{-6}). This variant RS2859144 (or proxy) was consistently associated with MPO in the FHS and CHS studies (replication meta-P=0.003), leading to a meta-P value of 9.96×10^{-7} in the 3 dyslipidemic groups. The variant or its proxy was then profiled in additional population-based cohorts (up to a total of 58 930 subjects)

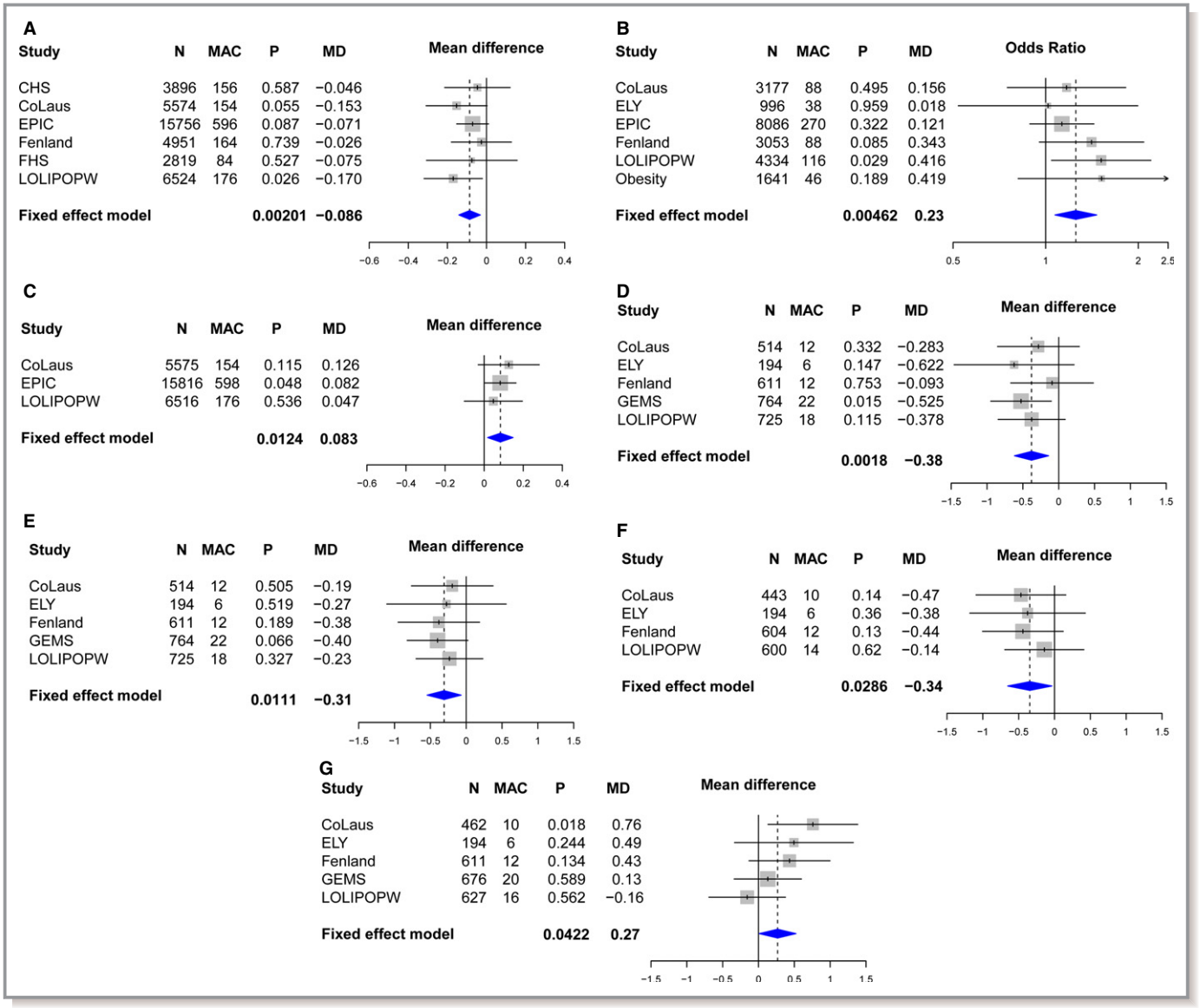

Figure 9. Association analyses for RS2859144/RS612049 with metabolic variables in overall (A) glomerular filtration rate, (B) obesity status, (C) uric acid and dyslipidemic populations, (D) systolic blood pressure, (E) diastolic blood pressure, (F) HOMA-B, and (G) glucose. CHS indicates Cardiovascular Health Study; CoLaus, Cohorte Lausannoise; EPIC, European Prospective Investigation of Cancer; FHS, Framingham Heart Study; GEMS, Genetic Epidemiology of Metabolic Syndrome Study; LOLIPOPW, London Life Sciences Prospective Population Study Whites; MAC, minor allele count; MD, mean difference; P, p value.

including CoLaus, Ely, Fenland, EPIC, LOLIPOP, and the GenOA study obesity case–control for up to 40 cardiovascular and metabolic traits highlighting the same SNP, which was also nominally associated consistently with GFR (P=0.002) and risk of obesity (BMI≥30 kg/m^2, P=0.004) in an overall analysis. BMI as a continuous trait was not nominally significant (P=0.052) and was not significant within the GIANT consortium,[22] suggesting either this is a false positive or specific to a more extreme form of obesity.

Deep sequencing identified only 1 low-frequency coding variant within *MAPK11* (rs33932986, R275H, MAF=0.02) and no others with more than a few copies. We observed only 1 low-frequency coding splice variant within *MAPK14* (rs200906391, European MAF=0.0067), and only 2 other variants were present with more than a few copies within Europeans (L250F and D343G). The function of the 2 rare variants observed in this study (L250F and D343G) has not been studied, though a PHYLOP score of >2 indicates that they are evolutionarily conserved. No robust associations were observed with these 2 variants, though a nominally significant rare variant association was observed with IL-6 levels in GEMS (Figure 11). Treatment with SB681323 (a back-up P38 MAPK inhibitor) demonstrated a trend for reduced IL-6 production 2 days after a percutaneous

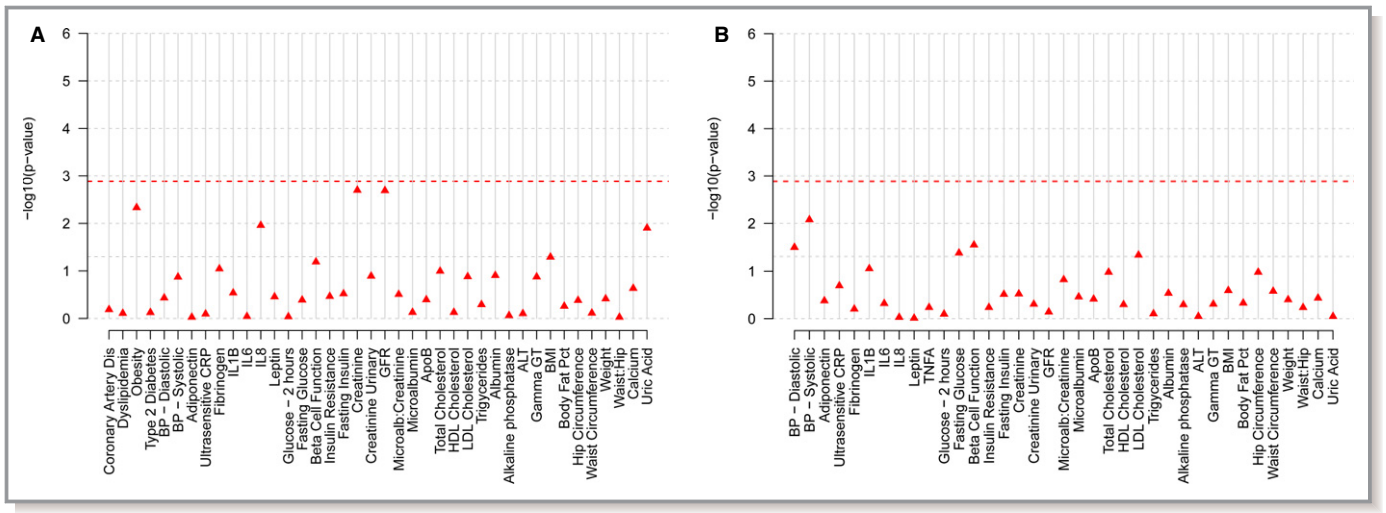

Figure 10. Analysis of cardiovascular and metabolic phenotypes in CoLaus, LOLIPOP, EPIC-Norfolk, ELY, Fenland, and GenOA studies. The figure summarizes the *P*-values for association tests performed for rs2859144 within the *MAPK14* gene for 40 cardiovascular and metabolic traits combining results in a meta-analysis from the CoLaus, LOLIPOP, EPIC-Norfolk, ELY, Fenland, and GenOA studies. Analyses were performed in all subjects (A—40 traits) and within dyslipidemic subjects (B—37 traits). For each trait, the *P*-values are shown. *P*-value thresholds are specified with a line and were calculated as 0.05/(no. of traits) ≈0.00125. ALT indicates alanine aminotransferase; ApoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; CoLaus, Cohorte Lausannoise; CRP, C-reactive protein; EPIC, European Prospective Investigation of Cancer; GFR, glomerular filtration rate; Gamma GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; LOLIPOP, Life Sciences Prospective Population Study.

intervention[1] and also significantly reduced IL-6 in rheumatoid arthritis patients after a single dose.[23]

The 3 low-frequency variants in high linkage disequilibrium that were found to be associated with MPO as well as obesity and GFR have not been functionally characterized. Two of these variants are within *MAPK14*, RS2859144 within the 3′untranslated region and RS2815805 within exon 9, RS612049 is 5′ to *MAPK14* and also falls within the first exon of *SLC26A8*, which is transcribed in the opposite direction (Figure 4). SLC26A8 is an anion transporter

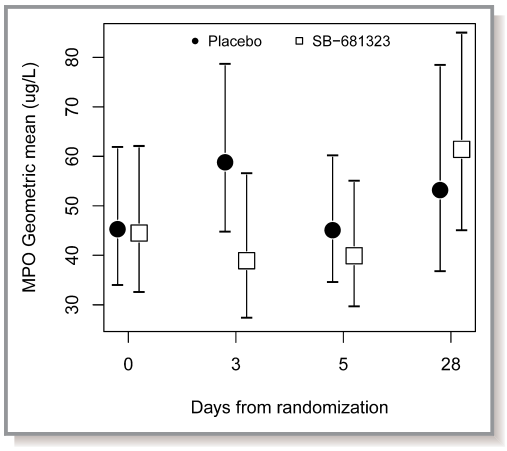

Figure 11. MPO plot from P38 *MAPK* inhibitor study. This figure is reproduced from data included in Sarov-Blat et al[1] showing a trend in post-stent dampening at day 3 in MPO levels by SB681323. MPO indicates myeloperoxidase.

expressed solely in the testes[24] and therefore is not likely to explain the effects observed here. We searched ENCODE data through the RegulomeDB portal[25] to see if there was any evidence for regulatory function of these SNPs. RS281505 showed only minimal binding evidence based on transcription factor binding and the presence of a DNase peak. Thus, the functionality underlying these associations remains to be determined. Lastly, according to genome wide association study central, RS612049 and other SNPs in linkage disequilibrium are close to genome-wide significant (minimum $P=3\times10^{-8}$) for height only.[26]

The association of MPO levels and the *MAPK14* variant provides valuable insight into the action of MAPK-α/β inhibitors. High MPO levels are an important prognostic marker in predicting coronary events in healthy individuals[21] and coronary artery disease patients[16,27] and thus aligned well with results obtained in the SOLSTICE study where a reduction in post-percutaneous intervention coronary events was observed in patients on Losmapimod.[3] Moreover, there is some precedent for the dependence on HDL cholesterol, as MPO has been observed to predict carotid stenosis only on a background of low HDL cholesterol.[28] MPO is a leukocyte-derived enzyme that catalyzes the formation of a number of reactive oxygen species, which contribute to tissue damage during inflammation. It selectively modifies apolipoprotein A-I, generating dysfunctional HDL cholesterol with impaired cholesterol efflux activity as well as generating pro-atherogenic forms of modified LDL cholesterol.[29] A major determinant of coronary plaque progression or regression rate is the

balance between cholesterol uptake versus efflux pathways, and excess MPO would be likely to impair both processes. The relationship between MPO and p38 map kinase was further supported by a trend in reduction of MPO post-percutaneous intervention with SB681323-treated patients compared to an increase in placebo patients (Figure 11).[1] Taken together, these results suggest a mechanistic link between P38 map kinase and MPO, thus providing evidence to support the current indication of acute coronary syndrome for Losmapimod. We could also hypothesize that patients with low HDL and/or high MPO levels might benefit more from Losmapimod treatment than those without such levels.

The same set of genetic markers that were associated with MPO also showed some suggestive associations with other traits in the overall sample. Lower GFR and increased risk of obesity as a binary trait exhibited directionally consistent associations across 6 datasets, although this was not statistically significant. The link between inflammation and obesity is well established; therefore, it is not surprising to observe an increased risk for obesity associated with the same allele linked with higher MPO, though the lack of a BMI association in the GIANT consortium might suggest that this is a false positive or related to more extreme obesity parameters. GFR is a commonly used clinical measure of kidney function, and evidence exists for an important pathogenic role for p38 MAPK activation in human glomerulonephritis.[30] Furthermore, protection of kidney function conferred by a p38 map kinase inhibitor has been observed in hypertensive preclinical models.[31] Thus, a p38 map kinase inhibitor would be predicted not only to have cardioprotective effects, but also a protective effect on the kidney and perhaps even generate some weight loss, though the latter would be unlikely with short-term use. Lastly, elevated uric acid levels could be interpreted as a nonspecific indicator of increased cardiovascular risk, which is not thought to be in itself causal.[32,33]

The variables nominally associated with the *MAPK14* variants within the dyslipidemia subgroup need to be interpreted with caution due to the modest significance levels and small number of carriers, but are provided for descriptive purposes. If they were to be confirmed in larger sample sizes, they would suggest a slightly worse metabolic syndrome phenotype in carriers as compared to noncarriers. The more general limitations of this report include the following: relatively small numbers of carriers due to the low frequency of the variant, limited power to detect associations with rare variants unless effects are large, data are restricted to populations of European origin, and the fact that the actual causal variant is not known.

In conclusion, by sequencing we have determined the full range of exonic variation, present in individuals of European ancestry, within *MAPK14* and *MAPK11* and tested for associations with a wide range of traits and conditions. We then followed up the most promising findings within up to 58 930 subjects. The most consistent associations observed were with a variant in *MAPK14* and MPO on a low HDL-C background and also more marginal associations with risk of obesity and kidney function in an overall analysis. These results also illustrate how mining of low-frequency variation may reveal useful tools for associating genes with phenotypes of interest, when common variation has not been informative. This study also illustrates the value of having good intermediate phenotypes and how extreme sampling, as in the case of GEMS, can reveal context-specific associations not observed in all-comers. However, the low-frequency variation does require access to substantial follow-up samples to evaluate the consistency of the data, especially in the present case where genetic effects were partly context dependent. Lastly, sequencing across multiple ethnic groups indicates that subjects of African origin would be of particular interest for further follow-up, as the RS2859144 and related markers are much more common in African-Americans (MAF 14%) as compared to subjects of European origin.

Acknowledgments

We thank the GSK sample preparation team, and D. Fraser for data preparation, and K. Johansson for consent review. We thank the GEMS Study Investigators: Philip Barter, PhD; Y. Antero Kesäniemi, PhD; Robert W. Mahley, PhD; Ruth McPherson, FRCP; and Scott M. Grundy, PhD. We also thank Dr Waeber MD, the CoLaus PI's Peter Vollenweider, MD and Gerard Waeber, MD, the LOLIPOP PI's, Jaspal Kooner, MD and John Chambers, MD, as well as the participants in all the studies. I would like to thank Vincent Mooser for his support for this work and valuable scientific discussions.

Sources of Funding

The genotyping and analysis at the University of Cambridge was supported in part by funding from GlaxoSmithKline under a collaboration agreement. The GEMS study was sponsored in part by GlaxoSmithKline. The CoLaus study was supported by grants from GlaxoSmithKline, the Swiss National Science Foundation (Grant 33CSCO-122661), and the Faculty of Biology and Medicine of Lausanne. Framingham Heart Study (FHS) research was conducted in part using data and resources of the National Heart, Lung, and BloodInstitute (NHLBI) and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the FHS investigators participating in the SNP Health Association Resource (SHARe) project. This work was partially supported by NHLBI (contract no. N01-HC-25195) and its contract with Affymetrix for genotyping services (contract no. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center.

Disclosures

Waterworth, Li, Warren, Aponte, Sarov-Blat, Sprecher, Chissoe, and Ehm are all full-time employees of GlaxoSmithKline.

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