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Genetic variation of the interleukin-1 family and nongenetic factors determining the interleukin-1 receptor antagonist phenotypes

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Abstract

The natural anti-inflammatory protein interleukin-1 receptor antagonist (IL-1Ra) inhibits the activity of IL-1 and is associated with vascular injury and metabolic disorders. We analyzed genetic and nongenetic determinants of the IL-1Ra phenotype. Fifteen haplotype-tagging single nucleotide polymorphisms (SNPs) in the IL-1α (*IL1A*), IL-1β (*IL1B*), and IL-1 receptor antagonist (*IL-1RN*) genes were determined in the Health 2000 survey (n = 6771) and European myocardial infarction (MI) survivors (n = 972). Three SNPs were genotyped in the FINRISK97 (FR97) study (n = 7222). We found 3 *IL1RN* variants that were associated with the IL-1Ra phenotype in the study populations and remained significant after Bonferroni correction with increasing significance in meta-analysis (*P* values for *rs3213448, rs315952, rs315949,* respectively: 5.5×10^{-11}, 1.5×10^{-11}, and 4.0×10^{-14}). Minor allele of the rare *IL1B* variant *rs1143642* was associated with decreased IL-1Ra levels in the Health 2000 and FR97 populations, and the association strengthened in the meta-analysis ($P = 9.4 \times 10^{-7}$). The proportion of variance explained by the *IL1RN* variant was larger in MI survivors (5.0%) than in the unselected population (0.5%). Body mass index was the strongest nongenetic predictor of the IL-1Ra phenotype, explaining 11.8% of the variance in Health 2000, 18.1% in FR97, and 25% in MI survivors. In conclusion, 3 *IL1RN* SNPs and 1 *IL1B* variant were determining IL-1Ra phenotype independently of body mass index and other metabolic phenotypes. The proportion of phenotypic variation in IL-1Ra explained by the genetic variants was, however, modest compared with the proportion explained by the body mass index.

1. Introduction

Interleukin-1 (IL-1) is an upstream cytokine family, which is emerging as an important contributor to the vascular injury and metabolic dysregulation. It includes proinflammatory cytokines IL-1α and IL-1β, and anti-inflammatory IL-1 receptor antagonist (IL-1Ra). Proinflammatory activation links IL-1β with the initiation of atherogenesis, lipid disorders, and activation of the pathways promoting vascular injury [1]. Increased expression and secretion from adipose tissue of the natural IL-1Ra have previously been shown to associate with obesity [2]. The blood levels of IL-1Ra were nearly 7-fold increased in morbidly obese patients when compared with lean control subjects, and the insulin resistance index was the most important determinant of the IL-1Ra level [3]. In the same study, a surgical intervention for obesity was followed by a significant decrease of the cytokine antagonist levels.

The systemic levels of IL-1Ra were increased and predicted the incidence of type 2 diabetes mellitus in a

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prospective case-control study of the Whitehall II cohort [4]. An attenuation of the association was observed after adjustment for 2-hour glucose and waist circumference. The clinical use of the IL-1 receptor blockade with synthetic IL-1ra anakinra was examined in a recent randomized double-blind trial. The results showed that IL-1Ra had an impact on glucose homeostasis, suggesting a possible therapeutic potential in the treatment of type 2 diabetes mellitus [5].

Earlier studies of the genetic variation in the IL-1 family have produced evidence that the IL-1Ra gene (*IL1RN*) variants may be associated with the IL-1Ra levels, and a quantitative trait locus was proposed in a genomewide association study [6,7]. Moreover, common *IL1RN* variants have been examined in 2 independent studies showing suggestive evidence for an association with markers of systemic inflammation [8]. In general, however, the genetic and nongenetic determinants of IL-1Ra are insufficiently known at the moment. In the present study, we investigated the genetic and nongenetic predictors of the IL-1Ra phenotype in 3 independent populations.

2. Methods

2.1. Study populations

The Health 2000 survey was based on a nationally representative population sample of 8028 men and women 30 years or older. Altogether, 6771 persons participated (84.3%) in the laboratory investigations and gave an informed consent [9]. A multicenter longitudinal study, "Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group" (AIRGENE), was conducted in 6 European cities—Athens, Augsburg, Barcelona, Helsinki, Rome, and Stockholm—between May 2003 and July 2004. The study recruited a total of 1003 myocardial infarction (MI) survivors and examined each subject in 6 clinical visits every 4 to 6 weeks. Interleukin-1 receptor antagonist was measured in 2 centers—Helsinki and Augsburg—including 2299 blood samples from 392 individuals. Full details of the study have been described previously [10]. The FINRISK97 study (FR97) is a population-based risk factor survey with 8444 participants carried out in 5 geographical areas in Finland. The age range was 25 to 74 years [11]. Baseline characteristics of the study populations are shown in Table 1. All projects have been approved by their respective ethics committees, and the participants gave a written informed consent.

2.2. Laboratory measurements

Interleukin-1 receptor antagonist was determined by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The interassay and intraassay coefficients of variations were 5.68% and 3.59%, respectively, for the FR97 and Health 2000 study populations. For the AIRGENE study population, the interassay coefficient of variation was 9.0% at an IL-1Ra concentration of 150 pg/mL, 9.7% at 300 pg/mL, 11.5% at 600 pg/mL, and 12.0% at 1400 pg/mL. Mean levels of the 6 follow-up visits were used for the IL-1Ra concentrations in the AIRGENE study, and the coefficients of variations for repeated IL-1Ra measurements ranged from 7.62% to 7.68% in each visit.

2.3. Selection and genotyping of single nucleotide polymorphisms

We selected common (> 5% minor allele frequency [MAF]) haplotype bin tagging single nucleotide polymorphisms (SNPs) in the IL-1α (*IL1A*), IL-1β (*IL1B*), and IL-1 receptor antagonist (*IL1RN*) genes from the Seattle SNP variation discovery database based on data available in

Table 1
Baseline characteristics of MI survivors (AIRGENE), Health 2000 study, and FR97 study populations

	AIRGENE	Health 2000	FR97
	n = 972	n = 6771	n = 7222
Sex (% male)	766 (78.8)	3011 (44.7)	3535 (49.0)
Age (y)	64.6 ± 9.4	52.8 ± 16.4	48.6 ± 13.4
BMI (kg/m^2)	28.4 ± 4.2	26.9 ± 4.9	26.6 ± 4.5
Smoking, n (%)	75 (7.7)	1480 (21.8)	1693 (23.5)
Total cholesterol (mmol/L)	4.77 ± 1.02	5.94 ± 1.12	5.54 ± 1.06
Systolic blood pressure (mm Hg)	136 ± 21.3	134 ± 34.4	136 ± 19.9
Hypertension, n (%)	495 (50.9)	1228 (18.1)[a]	3587 (49.7)
Diabetes, n (%)	191 (19.6)	595 (8.8)[b]	434 (6.0)
Medication for hyperlipidemia, n (%)	187 (19.2)	131 (1.9)	249 (3.5)
History of stroke, n (%)	59 (6.1)	358 (5.3)	162 (2.2)
History of MI, n (%)	972 (100)	204 (3.0)	226 (3.3)
IL-1Ra (pg/mL)[c]	238.0 (182.6-298.9)	326.5 (223.5-457.5)	245.5 (176.0-324.4)

Data are mean ± standard deviation unless otherwise indicated.

[a] Blood pressure greater than 140/90 mm Hg or on treatment.

[b] Including impaired fasting glucose and impaired glucose tolerance.

[c] Geometric mean and interquartile range are given for variables with skewed distributions.

Table 2
Selected SNPs in the *IL1A*, *IL1B*, and *IL1RN* genes and their MAFs in MI survivors (AIRGENE), Health 2000, and FR97 populations

Gene	Reference SNP ID	Alleles	Location	Seattle SNPs *literature alias*	MAF		
					AIRGENE	Health 2000	FR97
IL1A	rs3783521	G>A	Promoter	4521	0.317	0.38	
	rs1800587	G>A	5′ UTR	5138 *−889 C/T*	0.302	0.32	
	rs2856838	A>G	2nd intron	8126	0.377	0.29	
	rs3783546[a]	C>G	5th intron	13270	0.317	0.38	
	rs3783548	A>G	5th intron	14767	0.081	0.10	
IL1B	rs16944	G>A	Promoter	794 *−511C/T*	0.347	0.39	0.40
	rs1143629[a]	T>C	Intron	2143	0.348	0.39	
	rs3917356	C>T	Intron	3298	0.439	0.40	
	rs1143634	G>A	Coding exon	5277 *+3954 C/T*	0.246	0.26	
	rs1143640[a]	I>D	Intron	6909	0.246	0.26	
	rs1143642	G>A	Intron	7114	0.070	0.054	0.066
IL1RN	rs2637988	A>G	Intron	3331	0.422	0.47	
	rs3213448	G>A	Intron	5848	0.15	0.24	0.23
	rs315934	T>C	Intron	10 257	–	0.17	
	rs4252008	D>I	Intron	12 437	0.278	0.29	
	rs2232354	T>G	Intron	13 888	0.212	–	
	rs315952	T>C	Coding exon	16 857 *exon 6 30735*	0.302	0.42	
	rs4252041	C>T	3′ UTR	17163	0.041	0.02	
	rs315949	G>A	Downstream	19327	0.392	0.32	

D indicates deletion; I, insertion.
[a] Excluded from the further analysis because of complete LD ($r^2 \geq 0.99$) with another SNP in the study.

September 2005 as shown in Table 2 (pga.gs.washington.edu). According to the Seattle SNP database, the total number of TagSNPs in the IL-1 family is 102; and our set covers 100% of the haplotypic variation in *IL1A*, 100% in *IL1B*, and 87.5% in *IL1RN* with the r^2 threshold of 0.65 and MAF cutoff of 5%. The selected SNPs in the IL-1 genes cover 88 (19.2%) of the common variant SNPs in the HapMap data (Phase III, National Center for Biotechnology Information B36 assembly) region chr2:113259442-113608063 with the r^2 threshold of 0.80.

Genotyping was performed for 6461 individuals in the Health 2000, 972 in the AIRGENE and 6052 in the FR97 study population. During aliquotting, PicoGreen fluorescent label (Invitrogen, Molecular Probes, Carlsbad, CA) was used to quantify DNA concentration and to normalize the samples for genotyping. We used Sequenom MassARRAY system (Sequenom, San Diego, CA) for genotyping SNPs with homogenous mass extension protocol as specified by the manufacturer. The discordance rate was 0% in the genotyping sets including 2.2% known duplicate samples and 2.2% negative controls. In the FR97 study population, the genotyping was also carried out with Sequenom; but it included only 3 SNPs from the IL-1 family selected on the basis of Health 2000 and AIRGENE analyses. Genotyping success rate was greater than 95% for all SNPs. Three SNPs (*rs3783546*, *rs1143629*, and *rs1143640*) were excluded from the analysis because of complete linkage disequilibrium (LD) with *rs3783521*, *rs16944*, and *rs1143634*, respectively ($r^2 > 0.99$): 2 SNPs represented different haplotype bins in the Seattle SNPs database but were in complete LD in our sample, and 1 pair (*rs16944* and *rs1143629*) was purposely selected from the same haplotype bin to ensure the genotyping of *IL1B* −511C/T. The distribution of genotypes did not deviate from Hardy-Weinberg equilibrium (HWE) except in 1 case (*rs1143642*). The HWE *P* value was .014 in the Health 2000 population.

2.4. Haplotype construction and estimation

Haploview software version 3.32 was used to define haplotype blocks in the IL-1 genes [12]. In addition, the algorithm of Gabriel et al [13] was used to generate 2 subhaplotypes from *IL1RN*. Haplotypes were inferred using PHASE 2.0 software including only individuals with full genotype data [14]. Haplotypes were inferred after 40 iterations to minimize the number of ambiguous results. The *IL1B* SNP *rs1143642* that deviated from HWE was found to tag a single haplotype. The LD and correlation data plot of the *IL1RN* SNPs together with 2 *IL1A* and *IL1B* variants with MAF less than or equal to 0.1 in the Health 2000 study is shown in the Supplementary Figure 1.

2.5. Statistical methods

We used linear regression analysis based on additive models for the multivariate analyses and adjusted for age, sex, body mass index (BMI), total cholesterol to high-density lipoprotein (HDL) ratio, systolic blood pressure, current smoking, hypertension, and residential area. Interleukin-1 receptor antagonist was log-transformed, and geometric means are reported. The trends across the 3 genotypes were computed including effect per allele estimates. The proportions of IL-1Ra phenotypic variance explained by genetic and nongenetic factors were calculated. Replication of the results was evaluated in the 3 study populations, and the

Table 3
The multivariate-adjusted[a] associations of SNPs in *IL1A*, *IL1B*, and *IL1RN* genes with the IL-1Ra phenotype in the MI Survivors (AIRGENE), Health 2000 study, and FR97 study populations

SNP	MI survivors				Health 2000				FR97				Meta
	Genotype[b]	β	Effect[c]	P	Genotype[b]	β	Effect[c]	P	Genotype[b]	β	Effect[c]	P	P
IL1A													
rs3783521	38/185/159	−0.046	−12.66	.105	890/2740/2259	−0.004	−3.56	.661					.175
rs1800587	37/168/181	−0.043	−11.88	.123	632/2538/2720	0.022	7.34	.029					.062
rs2856838	45/163/160	0.084	23.46	.003	499/2460/2938	−0.018	−4.55	.082					.280
rs3783548[d]	0/68/313	−0.091	−23.33	.051	62/1067/4785	−0.036	−12.02	.033					.004
IL1B													
rs16944	56/162/169	0.006	2.03	.801	882/2822/2218	−0.002	−0.09	.830	1038/3038/2354	0.009	2.04	0.258	.223
rs3917356	78/158/133	0.009	4.24	.707	926/2800/2167	−0.014	−3.49	.151					.113
rs1143634	24/148/213	−0.029	−0.11	.320	403/2228/3262	0.023	3.21	.030					.044
rs1143642[d]	1/37/349	−0.049	−12.62	.410	29/575/5211	−0.059	−19.55	.007	0/864/6007	−0.063	−15.21	1.5×10^{-4}	1.1×10^{-6}
IL1RN													
rs2637988	96/192/99	0.012	3.15	.651	1287/2933/1676	−0.036	−12.30	1.1×10^{-4}					2.4×10^{-4}
rs3213448	15/131/241	0.052	34.89	.105	336/2151/3406	0.044	13.52	6.2×10^{-5}	336/2236/3636	0.046	11.10	2.1×10^{-6}	7.1×10^{-11}
rs315934					165/1624/4144	0.039	15.03	.002					
rs4252008	35/160/191	−0.025	−5.12	.368	496/2498/2936	0.006	3.54	.546					.401
rs2232354	11/108/267	−0.052	−16.05	.127									
rs315952	46/176/147	0.124	40.55	4.2×10^{-6}	1024/2843/1993	0.050	17.51	1.3×10^{-7}					2.8×10^{-11}
rs4252041[d]	1/24/362	−0.281	−65.44	1.0×10^{-4}	2/226/5706	−0.058	−19.30	.091					.001
rs315949	42/173/170	−0.107	−22.46	6.8×10^{-5}	596/2511/2782	−0.064	−20.15	1.0×10^{-10}					3.8×10^{-14}

The direction of the association is described with increasing number of minor alleles. Underlined *P* values remain significant after Bonferroni correction.
[a] Adjusted for age, sex, BMI, total cholesterol to HDL ratio, systolic blood pressure, current smoking, hypertension, and residential area.
[b] n (minor homozygotes/heterozygotes/major homozygotes).
[c] Effect per allele.
[d] Minor homozygotes and heterozygotes are pooled.

results were pooled using fixed-effect meta-analysis. We used Bonferroni correction to adjust for multiple testing, but nominal *P* values are also shown. The haplotypes were correspondingly analyzed by comparing the carriers of 2 copies of the haplotype with the carriers of 1 copy or no copy of the haplotype (or simple carrier analysis). Bonferroni correction was not done for haplotype analyses. The statistical analyses were carried out with SAS, version 9.1.3 (SAS Institute, Cary, NC).

3. Results

Four common variant SNPs in the *IL1RN* gene were associated with the IL-1Ra phenotype in multivariate analysis and remained significant after Bonferroni correction (Table 3). Associations of the *IL1RN* SNPs *rs3213448*, *rs315952*, and *rs315949* with the IL-1Ra phenotype were consistent and replicated in 2 or 3 study populations; and the statistical significance increased in the meta-analyses. The minor allele of intronic SNP *rs3213448* was associated with increased IL-1Ra levels, and the direction of the association was consistent in all 3 study populations. The synonymous coding variant *rs315952* was available in 2 populations. In both of them, the minor allele was associated with increased IL-1Ra levels. *rs315949*, located near 3′ UTR, was also available in 2 populations; and its minor allele was associated with decreased IL-1Ra level in both populations. The fourth intronic *IL1RN* SNP, *rs2637988*, was significantly associated with the IL-1Ra phenotype in the Health 2000 population; but the association was nonsignificant among the MI survivors.

The 3 SNPs from *IL1A*, *IL1B*, and *IL1RN* with MAFs less than or equal to 0.1 showed each consistent effect estimates and *P* values less than .004 in the meta-analysis for association with the IL-1Ra levels analyzed in 2 or 3 study populations (Table 3). The minor allele of the *IL1B* intronic rare variant *rs1143642* was associated with decreased IL-1Ra levels in the Health 2000 and FR97 populations. Among MI survivors, the association was not significant; but the direction was consistent with the 2 other study populations. Thus, the statistical association increased quite markedly in the meta-analysis ($P = 1.1 \times 10^{-6}$). Minor allele of the 3′ UTR *IL1RN rs4252041* was associated with decreased IL-1Ra levels among MI survivors only.

The proportion of variance of the IL-1Ra phenotype explained by single *IL1RN* SNPs *rs3213448*, *rs315952*, and *rs315949* ranged from 0.2% to 0.5% in the Health 2000 and FR97 populations and from 1.4% to 5.0% in MI survivors. The *IL1RN rs315952* was found to be the strongest genetic predictor for the IL-1Ra phenotypic variation in MI survivors and Health 2000 after adjustment for age and sex. The proportion of IL-1Ra variance explained by haplotypic variation of the *IL1RN* SNPs *rs315952*, *rs4252041*, and *rs315949* was 0.7% and 4.0% in Health 2000 and MI survivors, respectively (Table 4).

Table 4
The multivariate-adjusted[a] associations with increasing copy number of haplotypes (SNPs rs315952, rs4252041, and rs315949) in *IL1RN* genes with the IL-1Ra phenotype in the MI survivors (AIRGENE) and Health 2000 study

Haplotype	No/1/2 copies	MI survivors			No/1/2 copies	Health 2000		
		β	Proportion[b] (%)	*P* value		β	Proportion[b] (%)	*P* value
CCG	147/175/46	0.124	4.0	3.4×10^{-6}	2056/2944/1060	0.050	0.7	1.1×10^{-7}
TCA	179/156/33	−0.071	1.2	.011	3029/2490/541	−0.063	0.005	7.8×10^{-10}
TCG	178/157/33	−0.022	0.1	.42	3233/2409/418	0.010	0.2	.36
T*T*A	345/22/1	−0.276	2.6	2.0×10^{-4}	5823/235/2	−0.058	0.1	.093

[a] Adjusted for age, sex, BMI, total cholesterol to HDL ratio, systolic blood pressure, current smoking, hypertension, and residential area.
[b] Proportion of variance explained by the haplotype.

The IL-1Ra levels increased with age and were higher in women than in men (Table 5). Systolic blood pressure was positively associated with IL-1Ra in the healthy population samples. The presence of hypertension or diabetes was associated with higher IL-1Ra levels in all 3 study populations. By far, the strongest nongenetic predictors of IL-1Ra phenotype were BMI and waist circumference (Table 5). Body mass index explained 24.6%, 11.8%, and 18.1% of the phenotypic variation in MI survivors, Health 2000, and FR97, respectively, when adjusted for age and sex. Waist circumference was an even slightly stronger predictor than BMI, explaining 13.6% and 20.3% of the phenotypic variation of IL-1Ra in the Health 2000 and FR97 populations, respectively. The levels of glucose, insulin, triglycerides, and total cholesterol to HDL ratio also significantly explained the IL-1Ra variation in the Health 2000 study and FR97 populations.

Because obesity was the strongest nongenetic predictor of the IL-1Ra phenotype, we further analyzed whether the effects of the genetic variants described above could be mediated by BMI. To this end, we repeated the genetic association analyses by dropping BMI out from the covariates. This, however, led to attenuation, and not strengthening, of the genetic associations as shown in Supplementary Table 1.

4. Discussion

We studied 3 independent populations—2 representative general population samples and a sample of MI survivors of European descent—and found that the variation of the 3 common *IL1RN* variants—*rs3213448*, *rs315952*, and *rs315949*—was associated with the IL-1Ra phenotype after correction for multiple testing and adjusting for multiple covariates. For the *IL1B* SNP *rs1143642*, the very low number of minor allele homozygotes necessitated the use of a dominant model but also produced a clear statistical significance. Observed associations of the IL-1 gene variants with the IL-1Ra phenotype were independent of BMI. In fact, removing BMI among the covariates attenuated the genetic associations slightly, suggesting that they are not mediated by BMI.

The proportion of phenotypic variation in the IL-1Ra concentrations explained by the genetic variants was modest as compared with the proportion explained by BMI. Waist

Table 5
Nongenetic traits predicting the IL-1Ra phenotype in the 3 study populations

Trait	MI Survivors			Health 2000			FR97		
	β	Proportion[a] (%)	*P* value	β	Proportion[a] (%)	*P* value	β	Proportion[a] (%)	*P* value
Age (y)	−0.005	1.1	.038	0.003	0.6	7.9×10^{-10}	0.004	0.8	6.8×10^{-14}
Sex (reference: female)	−0.183	3.4	.0003	−0.121	1.2	2.9×10^{-18}	−0.139	1.8	9.4×10^{-29}
Total cholesterol to HDL ratio (mmol/L)	0.098	6.1	4.2×10^{-7}	0.068	3.9	6.7×10^{-57}	0.107	7.3	3.4×10^{-118}
BMI (kg/m^2)	0.048	24.6	1.8×10^{-26}	0.041	11.8	3.0×10^{-174}	0.052	18.1	$<1.1\times10^{-201}$
Systolic blood pressure (mm Hg)	0.001	0.3	.268	0.002	0.4	2.2×10^{-7}	0.003	0.7	$2.4e^{-12}$
Hypertension	0.080	0.9	.053	0.168	1.3	3.3×10^{-20}	0.135	1.4	3.0×10^{-23}
Current smoking	0.110	0.1	.644	0.091	0.4	1.1×10^{-7}	0.021	0.03	.166
Prevalent diabetes	0.162	2.3	.002	0.312	2.2	1.0×10^{-32}	0.194	0.7	3.1×10^{-13}
Waist (cm)				0.017	13.6	$<1.1\times10^{-201}$	0.021	20.3	$<1.1\times10^{-201}$
Insulin (pmol/L)				0.001	0.7	3.4×10^{-11}	0.013	4.6	3.3×10^{-72}
Glucose (mmol/L)				0.049	1.1	1.5×10^{-17}	0.024	0.3	9.7×10^{-5}
Triglycerides (mmol/L)				0.114	4.4	5.4×10^{-64}	0.126	6.1	1.9×10^{-99}

Age is adjusted for sex, and sex is adjusted for age. All other variables are adjusted for age and sex.
[a] Proportion of variance explained by trait.

circumference (indicating visceral obesity) and BMI were the strongest nongenetic determinants of the IL-1Ra phenotype. Total cholesterol to HDL ratio and triglycerides that were consistently predicting circulating IL-1Ra concentration were most likely consequential to the underlying visceral obesity, and the proportions of variance explained by these factors were lower than the proportions explained by BMI and waist circumference. The proportions of variance in the IL-1Ra phenotype explained by insulin and glucose were modest and comparable with the proportions explained by the genetic variants investigated in our study. The presence of hypertension or diabetes was consistently associated with higher IL-1Ra levels. Previously, Marculescu and coworkers [15] have reported, among patients investigated for suspected coronary artery disease, significantly lower IL-1Ra concentrations in those with diabetes than in those without diabetes. Our present results in all three study populations are at variance with these earlier findings. Reasons for the difference are not obvious but may be related to different patient characteristics. Nevertheless, it is possible that the increase of IL-1Ra observed in obese individuals may reflect an attempt to protect the body from the proinflammatory cytokines induced by obesity. If this response is insufficient, diabetes may develop. Prospective studies are of course needed to confirm this assumption.

Our previous analysis of the Health 2000 population revealed that persons homozygous for the *IL1RN rs3213448* minor allele had decreased fasting insulin concentration in addition to the increased IL-1Ra levels reported in this study. The *IL1B* minor allele *rs1143642* was associated with decreased 2-hour insulin in our earlier analysis and now with decreased IL-1Ra levels [16]. The most important association with fasting glucose and 2-hour glucose was observed for the *IL1B rs1143634*; but its association with the IL-1Ra phenotype in the present study was only marginal suggesting, that IL-1β may play a more important role in decreasing insulin-induced glucose transport [16]. The role of IL-1Ra is emerging in the development of type 2 diabetes mellitus, as IL-1β has been reported to inhibit insulin receptor substrate−1 expression mainly through messenger RNA, the amount of which was dependent on extracellular receptor kinase pathway, and also by a posttranscriptional mechanism independent of extracellular receptor kinase [17]. However, human pancreatic β-cells can express IL-1Ra; but the expression is decreased in patients with type 2 diabetes mellitus [18]. Accordingly, circulating systemic IL-1Ra levels may not necessarily reflect the local situation in pancreas [18].

A genomewide family-based linkage study of African Americans reported a strong candidate region for type 2 diabetes mellitus in chromosome 2 (logarithmic odds–LOD score 4.53) extending from 41 to 121 Mb and including candidate genes in IL-1 and IL-1 receptor families [19]. However, no IL-1 gene variants were reported in a newly published large-scale meta-analysis of genomewide data for continuous diabetes-related traits in nondiabetic participants, where different genetic architecture for β-cell function and insulin resistance was suggested [20]. Only a few variants showed significant associations with insulin resistance as compared with several variants associating with β-cell function. Overall, the role of IL-1 gene variation and IL-1Ra has been shown to be biologically plausible in multiple studies, thus gradually unveiling the role of the IL-1 family in the development of type 2 diabetes mellitus.

Association of the *IL1RN* variation with the IL-1Ra phenotype has been previously reported from a genomewide association study in an elderly Italian population from Tuscany [7]. Rafiq and coworkers [6] have reported an association of the common variant *IL1RN rs4251961* with the IL-1Ra phenotype, together with a suggestive association with metabolic traits. This variant was tagging the same haplotype (r^2 threshold of 0.65) as *rs315949* investigated in our study and was found to be associated with lower IL-1Ra levels. The *rs4251961* was also associated with lower IL-1Ra expression after peptidoglucan stimulation of whole blood samples in 285 healthy persons recruited from the metropolitan Seattle area in the United States [8].

The *IL1RN* haplotypes have also been investigated in another cohort of European MI survivors, suggesting an association of *rs2232354*, *rs315952*, and *rs315949* with the IL-1Ra messenger RNA levels [21]. This is well in line with the present study, where *rs315952* was associated with increased IL-1Ra level in MI survivors and in the healthy population sample. In an analysis of the Coronary Artery Risk Development in Young Adults (CARDIA) study *rs1143642* was marginally associated with the change in C-reactive protein levels between the ages 7 and 15 years in obese black individuals; and a corresponding association was also found in the haplotypic analysis of obese white and black participants [22].

Other studies of IL-1 gene variation have concentrated mainly on *IL1B* variation and its association with cardiovascular morbidity and nonmetabolic mechanisms underlying the association. A case-control study in patients younger than 50 years suggested an association of the *IL1B rs16944* minor allele with atherothrombotic events [23]. However, *rs16944* was not found to be a determinant for IL-1Ra phenotype in our study. Another nested case-control study of US physicians aged 40 to 84 years found very little evidence for an association of variation in the IL-1 gene family with MI or stroke [24]. Only *rs1143623* of *IL1B* showed a modest association toward reduced risk of MI.

Experimental studies have linked IL-1 cytokines to vascular injury. A mouse model using high fat feeding suggested that genetic deletion of the IL-1 receptor type I or administration of the IL-1Ra may inhibit atheroma formation and a rise in blood pressure in response to the environmental stimulus [25]. Indeed, IL-1β seems to cause neointimal thickening of the arterial wall; and IL-1Ra may prevent such occlusive vascular responses to injury [26]. In an experimental model for atherosclerosis, sustained IL-1Ra

depletion or overexpression has had potentially important effects on lipoprotein metabolism and foam-cell lesion development [27]. Taken together, the available data provide suggestive but insufficient evidence for association of the IL-1 genetic variation with atherosclerosis or atherothrombotic complications. However, the role of the *IL1RN* variation has not been thoroughly examined.

To our knowledge, this is the largest study examining the IL-1Ra phenotype and IL-1 gene variation in representative and carefully phenotyped population samples. We carried out detailed analyses of central cardiovascular risk factors and metabolic traits and established their associations with the IL-1Ra phenotype in 3 independent study populations. Furthermore, we identified genetic variants that were robustly associated with the IL-1Ra phenotype after adjusting for multiple nongenetic covariates and correcting for multiple testing. A limitation of the study was that it was based on the candidate gene approach. A genomewide study would have provided a more comprehensive picture of the genetic background of the IL-1Ra phenotype. The cross-sectional design of the study prevents causal conclusions and did not enable an analysis of gene-environment interactions.

In conclusion, we detected 3 variants of the *IL1RN* gene and 1 in the *IL1B* gene that were independent determinants of the IL-1Ra phenotype in 2 or 3 separate populations. These associations were not mediated by obesity, which was the strongest nongenetic predictor of circulating IL-1Ra concentration. The proportion of variance in IL-1Ra concentration explained by the 4 SNPs was statistically significant but modest in magnitude compared with the proportions explained by BMI and other metabolic traits. Further studies in this field may improve our understanding of the biological pathways linking inflammation, obesity, and glucose and insulin metabolism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2010.01.017.

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