REVIEW

Comparison of Effect Sizes Associated With Biomarkers Reported in Highly Cited Individual Articles and in Subsequent Meta-analyses

John P. A. Ioannidis, MD, DSc
Orestis A. Panagiotou, MD

Context Many biomarkers are proposed in highly cited studies as determinants of disease risk, prognosis, or response to treatment, but few eventually transform clinical practice.

Objective To examine whether the magnitude of the effect sizes of biomarkers proposed in highly cited studies is accurate or overestimated.

Data Sources We searched ISI Web of Science and MEDLINE until December 2010.

Study Selection We included biomarker studies that had a relative risk presented in their abstract. Eligible articles were those that had received more than 400 citations in the ISI Web of Science and that had been published in any of 24 highly cited biomedical journals. We also searched MEDLINE for subsequent meta-analyses on the same associations (same biomarker and same outcome).

Data Extraction In the highly cited studies, data extraction was focused on the disease/outcome, biomarker under study, and first reported relative risk in the abstract. From each meta-analysis, we extracted the overall relative risk and the relative risk in the largest study. Data extraction was performed independently by 2 investigators.

Results We evaluated 35 highly cited associations. For 30 of the 35 (86%), the highly cited studies had a stronger effect estimate than the largest study; for 3 the largest study was also the highly cited study; and only twice was the effect size estimate stronger in the largest than in the highly cited study. For 29 of the 35 (83%) highly cited studies, the corresponding meta-analysis found a smaller effect estimate. Only 15 of the associations were nominally statistically significant based on the largest studies, and of those only 7 had a relative risk point estimate greater than 1.37.

Conclusion Highly cited biomarker studies often report larger effect estimates for postulated associations than are reported in subsequent meta-analyses evaluating the same associations.

JAMA. 2011;305(21):2200-2210 www.jama.com

MANY NEW BIOMARKERS ARE continuously proposed[1-3] as potential determinants of disease risk, prognosis, or response to treatment. The plethora of statistically significant associations[4,5] increases expectations for improvements in risk appraisal.[6] However, many markers get evaluated only in 1 or a few studies.[7] Among those evaluated more extensively, few reach clinical practice.[8]

This translational attrition requires better study. Are the effect sizes proposed in the literature accurate or overestimated?[9] It is interesting to address this question in particular for biomarker studies that are highly cited. Many of these risk factors are also evaluated in meta-analyses[10] that allow overviews of the evidence. However, some meta-analyses may suffer bias from selective reporting, especially among small data sets[11-13]; then large studies may provide more unbiased evidence.

Here, we examined biomarkers that had been evaluated in at least 1 highly cited study and for which at least 1 meta-analysis had been performed for that same association. We aimed to compare the effect size of these associations in the most highly cited studies vs what was observed in the largest studies and the corresponding meta-analyses.

For editorial comment see p 2229.

METHODS

We considered biomarkers that had a relative risk (RR) estimate presented numerically in the abstract of an article that had received more than 400 citations in ISI Web of Science until December 2010. The threshold of 400 citations was decided a priori, to target approximately the top 3% of biomarker studies published in influential journals. Of those, we focused further on biomarkers with published meta-analyses on the same asso-

Author Affiliations: Stanford Prevention Research Center, Department of Medicine, and Department of Health Research and Policy, Stanford University School of Medicine, Stanford, California (Dr Ioannidis); and Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece (Drs Ioannidis and Panagiotou).
Corresponding Author: John P. A. Ioannidis, MD, DSc, Stanford Prevention Research Center, Stanford University School of Medicine, Stanford University, Medical School Office Bldg, Room X306, 251 Campus Dr, Stanford, CA 94305 (jioannid@stanford.edu).

ciation. Eligible RR metrics included risk ratios, odds ratios, and hazard ratios. Log odds scores were excluded.

We considered all markers of disease risk, prognosis, or treatment response representing body fluid, tissue, or imaging measurements. We did not consider demographic, anthropometric, social, environmental, psychological, and behavioral factors unless represented by a fluid, tissue, or imaging measurement. We did not consider associations with other markers and nonclinical outcomes.

Meta-analyses were eligible if they had addressed the same association (same marker, same outcome) as the one highlighted in the abstract of the highly cited study. When several eligible RR estimates appeared in the abstract of the highly cited study, we selected the one presented first.

Search Strategy

Screening citation counts for all biomarker studies is extremely difficult because there are probably more than 100 000 studies published to date. Instead, we focused our searches on 24 nonreview biomedical journals that receive very high numbers of citations (per 2009 Journal Citation Reports) and that may publish on new biomarkers. These journals are (in decreasing total citations) *Nature, PNAS, Science, New England Journal of Medicine, Cell, Lancet, Circulation, Cancer Research, Blood, Journal of Immunology, JAMA, Journal of Clinical Oncology, Journal of Clinical Investigation, Neurology, BMJ, Nature Genetics, Journal of Experimental Medicine, Journal of Clinical Endocrinology and Metabolism, Journal of the American College of Cardiology, Cancer, Gastroenterology, Clinical Cancer Research, Pediatrics,* and *Journal of the National Cancer Institute* (*JNCI*).

We searched for potentially eligible highly cited studies in ISI Web of Science (last update December 26, 2010) using the search *risk ratio OR relative risk OR odds ratio OR hazard NOT random**. The last item aimed to exclude randomized trials, which use RRs predominantly for treatment effects rather than biomarker associations. When 2 or more highly cited articles from the same cohort or study were identified, we included only the one published earlier. To test the sensitivity of the search strategy, we also searched without any journal limits for articles published in 2002: 9326 items were retrieved for that calendar year alone, vs 696 with the search limited to the 24 selected journals; the journal-unlimited search yielded 12 eligible highly cited biomarker studies, 10 of which (83%) were from the 24 journals.

For each potentially eligible highly cited biomarker study, we searched PubMed (last update January 3, 2011) for meta-analyses of the selected association highlighted in the abstract of the highly cited study (same biomarker and outcome). The search used the biomarker name and synonyms, limited to publication type = "meta-analysis." Whenever multiple eligible meta-analyses existed, we identified whichever included the most studies. In each meta-analysis, we also identified the largest study (the one with greatest weight [smallest variance]).

Data Extraction

For each highly cited study, we extracted information on journal, publication year, number of citations, and information on the selected RR: the biomarker of interest, condition/outcome implicated, sample size and number of outcome events, RR estimate, and corresponding exposure contrast.

For each meta-analysis, we extracted information on the journal; first author; total sample size and number of events; sample size and number of events in the largest study; the years of the published studies; summary RR estimate in the meta-analysis and 95% confidence interval (CI) by random effects[14] (or fixed-effect calculations, if random effects were not presented at all); RR in the largest study and its 95% CI; RR in the index highly cited study and its 95% CI, as given in the meta-analysis; and type of RR and exposure contrast used in the meta-analysis. When RR estimates and CIs were only shown graphically in forest plots, we perused the corresponding primary study publications. If this remained unclear, we used the open-source software Engauge Digitizer (version 4.1) to extract numbers from the graphically presented information.

Biomarkers are sometimes tested independently, but their true clinical value is best appreciated when one can document whether they confer incremental information beyond what other variables and other biomarkers can provide. We examined all the evaluated highly cited studies and all the respective meta-analyses to record how many used unadjusted effects, how many provided adjusted effects adjusting for factors other than biomarkers, and how many adjusted also for other biomarkers.

Finally, we noted whether the RR estimate and 95% CI for the highly cited study as extracted from each meta-analysis were identical to those reported in the abstract of the highly cited study. Whenever not identical, we noted whether this was due to differences in exposure contrast, adjusting covariates, or sample size (a larger or smaller data set included in the meta-analysis than in the original highly cited publication). For comparison against the largest study and the meta-analysis results, we used for consistency the RR estimate of the highly cited study as reported in the meta-analysis. Whenever a meta-analysis did not include the highly cited study, it was not considered eligible, because it addressed potentially a different question than the highly cited study. However, we accepted as eligible those meta-analyses that (1) had replaced the highly cited study in their calculations with data from a larger data set that included the data from the highly cited study; (2) had excluded the highly cited study because of some technical issue (eg, Hardy-Weinberg violation in a genetic study), but the data addressed the same question; or (3) presented collaborative meta-analyses of individual participant data that did not have the raw data from the highly cited study even though it addressed the same question. In these 3 cases, for comparison against the larg-

est study and meta-analysis result, we used the RR estimate of the highly cited study as reported in its original abstract. When the exposure contrast of categorical variables was different between the highly cited study and the corresponding meta-analysis, the RR was approximated for the same contrast as used in the meta-analysis, after converting exposure contrasts to standard deviation equivalents.

Data extraction was performed independently by 2 investigators. Discrepancies were discussed to reach consensus.

Analysis

We compared the magnitude of the effect sizes (RRs) in the highly cited study against the meta-analysis and against the largest study using the same exposure contrast. Exposures were coined consistently to represent RR values greater than 1.00 in the highly cited study, eg, if a highly cited study gave an RR of 1.5 for above vs below median values of the biomarker, this was coined to become an RR of 0.67 for below vs above median values of the biomarker. We estimated for how many associations the RRs were in opposite direction, larger, more than twice as large, more than 4 times as large, or different beyond chance in the highly cited vs the largest study and in the highly cited study vs the meta-analysis. We also calculated the ratio of the RR estimates (relative relative risk [RRR]) and their 95% CIs. All analyses were conducted in Stata version 10.1 (Stata Corp, College Station, Texas). All *P* values are 2-tailed.

RESULTS

Among 14 025 articles, 377 had received more than 400 citations. Of those, we identified 113 highly cited biomarker studies that listed at least 1 RR for a biomarker in the abstract.

Of the 113 studies, 13 were meta-analyses by themselves and 100 were primary studies. We identified published systematic reviews (n=44) that could potentially correspond to 61 of the 100 associations. On further scrutiny, 26 associations were excluded, because the RR in the abstract was possibly wrong and could not be identified in the full text of the highly cited article (n=1), systematic reviews did not provide any summary estimate for the eligible association (n=6), another highly cited study from the same population cohort had been published earlier (n=1), the highly cited study addressed different risk factors (n=6) or different outcomes (n=3) or had a different design (n=4) from the studies considered eligible for the meta-analysis, or no study-specific RRs could be retrieved from the meta-analysis (n=5). Therefore, 35 highly cited studies[15-49] published between 1991 and 2006 remained eligible (TABLE 1). For each of 2 associations (C-reactive protein and coronary heart disease, *Helicobacter pylori* and gastric cancer) there were 3 eligible highly cited studies; thus, 31 independent meta-analyses[50-80] were considered.

The 35 eligible highly cited articles had received a median of 645 (interquartile range [IQR], 526-1054) citations vs 609 (IQR, 503-804) for the 65 excluded articles (*P*=.83). The median publication year of eligible articles was 1996 (IQR, 1995-2000) vs 1998 (IQR, 1995-2001) for those excluded (*P*=.07).

Study and Meta-analysis Characteristics

The highly cited biomarkers (Table 1) included genetic risk factors (n=11 associations), blood proteins (n=3), other blood biomarkers (n=8), infectious agent biomarkers (n=6), and others

Table 1. Characteristics of the 35 Eligible Highly Cited Studies

Source	Condition or Outcome	Risk Factor	Sample Size (Events), No.	Relative Risk (95% CI)	Type of Relative Risk (Exposure Contrast)	Citations, No.
Giovannucci et al,[16] 1997	Prostate cancer	Androgen receptor gene, CAG repeats	1182 (592)	1.52 (0.92-2.49)	OR (≤18 vs ≥26 CAG repeats)	493
Chan et al,[20] 1998		IGF-1 levels	304 (152)	4.32 (1.80-10.6)	RR (highest vs lowest quartile)	1202
Forman et al,[21] 1991	Gastric cancer	*H pylori*	145 (29)	2.77 (1.04-7.97)	OR (exposed vs nonexposed)	968
Parsonnet et al,[22] 1991		*H pylori*	400 (200)	3.60 (1.80-7.30)	OR (exposed vs nonexposed)	2458
Nomura et al,[23] 1991		*H pylori*	218 (109)	6.00 (2.10-17.3)	OR (exposed vs nonexposed)	1347
Blaser et al,[48] 1995		*H pylori*, anti-cagA antibodies	206 (103)	1.90 (0.90-4.00)	OR (exposed vs nonexposed)	864
Ford et al,[41] 1994	Colon cancer	*BRCA1* gene, mutation carrier	1327 (699)	4.11 (2.36-7.15)	OR (carriers vs noncarriers)	1038
Ma et al,[47] 1999	Colorectal cancer	IGF-1 levels	518 (193)	2.51 (1.15-5.46)	RR (highest vs lowest quintile)	599
Ma et al,[44] 1997		*MTHFR* gene, C677T	528 (202)	0.49 (0.27-0.87)	OR (Val/Val vs Val/Ala or Ala/Ala)	499
Bell et al,[45] 1993	Bladder cancer	*GSTM1* gene, 0/0 genotype	440 (229)	1.70 (1.20-2.50)	OR (0/0 vs +/0 or +/+)	533
Hankinson et al,[40] 1998	Breast cancer	IGF-1 levels	1017 (397)	0.85 (0.53-1.39)	OR (top third vs bottom quintile)	1024

(continued)

Table 1. Characteristics of the 35 Eligible Highly Cited Studies (continued)

Source	Condition or Outcome	Risk Factor	Sample Size (Events), No.	Relative Risk (95% CI)	Type of Relative Risk (Exposure Contrast)	Citations, No.
Wolff et al,[46] 1993		DDE levels	229 (58)	4.08 (1.49-11.2)	OR (90th vs 10th percentile)	544
Toniolo et al,[43] 1995		Total estradiol levels	381 (130)	1.80 (0.80-3.80)	OR (highest vs lowest quartile)	406
Braun et al,[34] 2000	Breast cancer survival	Bone marrow micrometastasis	552 (71)	4.17 (2.51-6.94)	HR (present vs absent)	526
Ozaki et al,[15] 2002	Myocardial infarction	Lymphotoxin alpha gene, LTA exon1 10G>A	2139 (1133)	1.78 (1.39 – 2.27)	OR (AA vs GG+GA genotypes)	407
Pischon et al,[39] 2004		Adiponectin levels	798 (266)	0.39 (0.23-0.64)	RR (highest vs lowest quintile)	645
Stampfer et al,[42] 1992		Hyperhomocysteinemia	542 (271)	3.10 (1.40-6.90)	OR (high vs normal levels)	1193
Ridker et al,[24] 2000	Coronary heart disease	CRP levels	366 (122)	1.50 (1.10-2.10)	RR (highest vs lowest quartile)	2379
Danesh et al,[25] 2004		CRP levels	6428 (2459)	1.45 (1.25-1.68)	OR (top third vs bottom third)	1054
Danesh et al,[26] 2000		CRP levels	1533 (507)	2.13 (1.38-3.28)	OR (top third vs bottom third)	791
Després et al,[28] 1996	Ischemic heart disease	Hyperinsulinemia	196 (105)	1.70 (1.30-2.40)	OR (per SD)	1092
Lindpaintner et al,[29] 1995		ACE gene, deletion-insertion polymorphism	3590 (1250)	1.07 (0.96-1.19)	OR (DD vs DI vs II genotypes)	723
Weiss et al,[35] 1996	Coronary thrombosis	Glycoprotein IIIa gene, PI^{A2} polymorphism	139 (71)	2.80 (1.20-6.40)	OR (PI^{A1}/PI^{A2} or PI^{A2}/PI^{A2} vs PI^{A1}/PI^{A1} genotypes)	496
Clarke et al,[27] 1991	Vascular disease	Hyperhomocysteinemia	150 (123)	27.7 (3.20-240)	OR (exposed vs nonexposed)	1436
den Heijer et al,[30] 1996	Deep vein thrombosis	Hyperhomocysteinemia	538 (269)	2.50 (1.20-5.20)	OR (exposed vs nonexposed)	694
Poort et al,[49] 1996	Venous thrombosis	Prothrombin gene, G20210A	900 (426)	2.80 (1.40-5.60)	OR (AG vs GG or AA genotypes)	1903
Grant et al,[17] 2006	Type 2 diabetes	*TCF7L2* gene, DG10S478	3774 (1774)	1.45 (1.41-1.73)	RR (carriers vs noncarriers)	579
Deeb et al,[19] 1998		*PPARG2* gene, Pro12Ala	300 (91)	4.35 (1.24-15.3)	OR (Pro/Pro vs Pro/Ala and Ala/Ala)	665
Higashi et al,[36] 2002	Life-threatening bleeding with warfarin	*CYP2C9* gene, *2/*3 polymorphism	185 (32)	2.39 (1.18-4.86)	HR (*2 or *3 vs *1)	442
Enomoto et al,[31] 1996	Interferon response in HCV infection	$NS5A_{2209-2248}$ protein mutations	84 (63)	5.30 (1.60-18.0)	OR (per 1 amino acid change)	613
Pallares et al,[32] 1995	Pneumococcal pneumonia mortality	Penicillin resistance	504 (140)	1.0 (0.50-1.90)	OR (penicillin-resistant vs nonresistant strains)	556
Hillier et al,[33] 1995	Preterm delivery of low-birth-weight infant	Bacterial vaginosis	10 397 (504)	1.40 (1.10-1.80)	OR (exposed vs nonexposed)	542
Hageman et al,[18] 2005	Age-related macular degeneration	*CFH* gene, H1 haplotype	1360 (954)	2.46 (1.95-3.11)	OR (haplotypic)	588
Siris et al,[37] 2001	Fracture	Bone mineral density	163 979 (NR)	4.03 (3.59-4.53)	HR (osteoporosis vs normal)	452
Kuipers et al,[38] 1995	Atrophic gastritis and intestinal metaplasia	*H pylori*	107 (18)	9.00 (1.90-41.3)	OR (exposed vs nonexposed)	476

Abbreviations: CI, confidence interval; CRP, C-reactive protein; DD, homozygous for the deletional (D) allele; DDE, l,l-dichloro-2,2-bis(p-chlorophenyl) ethylene; DI, heterozygous for the D and I alleles; HR, hazard ratio; HCV, hepatitis C virus; *H pylori*, *Helicobacter pylori*; IGF-1, insulinlike growth factor 1; II, homozygous for the insertional (I) allele; NR, not reported; $NS5A_{2209-2248}$, amino acid sequence 2209 to 2248 of nonstructural protein 5A; OR, odds ratio; RR, risk ratio.

(n=7). Cancer-related (n=14) and cardiovascular-related (n=12) outcomes predominated. The median sample size for the 35 highly cited studies was 518 (IQR, 218-1327), and median number of events was 197 (IQR, 103-504). The median RR was 2.50 (IQR, 1.70-4.08). Diverse exposure contrasts were involved (Table 1). The 35 studies were published in 10 different journals (*New England Journal of Medicine*, n=13 studies; *JAMA*, n=4; *JNCI*, n=4; *Nature*, n=3; *Lancet*, n=3; other, n=8).

Thirty-one of the 35 associations were statistically significant, while 4 were not (*ACE* deletion-insertion polymorphism and ischemic heart disease,[29] penicillin resistance and pneumococcal pneumonia mortality,[32] insulinlike growth factor 1 levels and breast cancer in postmenopausal women,[40] and total estradiol levels and breast cancer[43]). In 3 of these 4, other statistically significant associations were also reported in the abstract of the same highly cited study.

TABLE 2 shows the characteristics of the meta-analyses corresponding to the eligible highly cited studies. These meta-analyses, published between 1998 and 2010, included a median of 24 (IQR, 12-42) primary studies. Five were meta-analyses of individual patient data.[57,65,68,71,74] For 18 associations, the highly cited was published early (within the first 2 years in the accumulation of evidence), while in the other 17 it was published later (median, 6 years [IQR, 5-13] in the accumulation of evidence). The median reported sample size was 12 128 (IQR, 4267-30 650); median number of events was 4790 (IQR, 2862-10 451).

The median sample size of the largest studies was 1820 (IQR, 721-5457) and median number of events was 509 (IQR, 123-1121). In 3 cases (C-reactive protein levels and coronary heart disease,[25] $NS5A_{2209-2248}$ [amino acid sequence 2209 to 2248 of nonstructural protein 5A] and interferon response in hepatitis C virus infection,[31] and bone mineral density and fracture[37]), the largest study was the highly cited one. Excluding these 3 cases, the median number of citations in the largest studies was only 79 (IQR, 34-159). The largest studies were published a median of 5 years (IQR, 2-8) after the highly cited study, but in 3 cases they were published before the highly cited studies.

Adjusting Factors

For 15 of the 35 studies, the biomarker effect was assessed in unadjusted analyses, and in the other 20 it was adjusted for other variables; in 7 of these studies, the adjusting variables also included other biomarkers. Similarly, for the meta-analyses, adjustments for other variables occurred in 21 cases and 7 included also other biomarkers (eTable, available at http://www.jama.com).

Comparison of Effect Sizes

TABLE 3 shows comparatively the effect sizes in each highly cited study, meta-analysis, and largest study. Of note, in 23 of the 35 associations, there were some differences between the RR reported in the original highly cited study and how it was represented in the meta-analysis in terms of exposure contrast (n=18), adjusting covariates (n=9), and/or sample size (n=7). However, the difference was equally likely to yield a smaller or larger estimate in the meta-analysis representation of the study results than in the original abstract of the highly cited study.

For 30 of the 35 associations (86%), the highly cited studies had a stronger effect estimate than the largest study, for 3 the largest and highly cited study coincided, and only twice was the effect estimate stronger in the largest than in the highly cited study (FIGURE). The RR estimate was in the opposite direction in the highly cited than in the largest study in 5 associations, and the increase was more than 2-fold greater in another 20 associations (more than 4-fold in 13 associations). Both early- and late-published highly cited studies showed more extreme results than those found in meta-analyses of these biomarkers (eFigure 1). Differences were beyond chance (RRR 95% CIs excluding 1.00) in 9 associations: 5 for which the highly cited study was published early (in the first 2 years of accumulation of published evidence) and 4 for which the highly cited study was published late. Of the 9 discrepancies, 3 involved highly cited studies for which the effect sizes had been adjusted for other variables.

For 29 of the 35 highly cited studies (83%), the corresponding meta-analysis found a smaller effect (Figure). The RR estimate was in the opposite direction in the highly cited than in the meta-analysis in 4 associations and the increase was more than 2-fold greater in another 14 associations (more than 4-fold in 7 associations). Both early- and late-published highly cited studies showed larger effects than those in meta-analyses (eFigure 2). Differences were beyond chance in 11 associations: 8 in which the highly cited study was published early (in the first 2 years of accumulation of published evidence) and 3 in which the highly cited study was published late. Of the 11 discrepancies, 6 involved highly cited studies where the effect estimates had been adjusted for other variables.

Only 15 associations were nominally statistically significant based on the largest studies and of those 7 had a point estimate RR greater than 1.37. Thirty-two of the 35 associations showed nominally statistically significant increased risk based on the meta-analyses and of those 18 had a point estimate RR greater than 1.37.

COMMENT

This empirical evaluation of 35 top-cited biomarker studies suggests that many of these highlighted associations are exaggerated. In some cases, these markers may have no predictive ability, if one trusts the subsequent replication record, in particular the results of the largest studies on the same associations. Less than half of these biomarkers have shown nominally significant results in the largest studies that have been conducted on them, and only 1 in 5 has shown an RR greater than 1.37. There are several true associations, but they correspond predominantly to small or modest effects with uncommon exceptions. Such effects, even if genuine, may

Table 2. Characteristics of the Corresponding Meta-analyses and Largest Studies

		Meta-analysis				Largest Study		
Disease or Outcome	Risk Factor	No. of Studies	Years of Published Studies	Sample Size (Events), No.	Reference	Sample Size (Events), No.	Year of Publication	Reference
Prostate cancer	Androgen receptor gene, CAG repeats	22	1997-2003	NR	51	552 (162)	1999	81
	IGF-1 level	42	1993-2007	19 347 (7481)	55	2691 (524)	2006	82
Gastric cancer	*H pylori*[a]	42	1990-1998	12 128 (4241)	56	1651 (243)	1994	83
	H pylori, anti-cagA antibodies	13	1995-2003	2980 (1466)	79	392 (162)	2003	84
Colon cancer	*BRCA1* gene, mutation carrier	36	1985-2004	NR	72	145 677 (861)	2001	85
Colorectal cancer	IGF-1 levels	11	1999-2010	7828 (2862)	78	2242 (1121)	2010	78
	MTHFR gene, C677T	29	1996-2008	30 650 (11 936)	75	4349 (2178)	2004	86
Bladder cancer	*GSTM1* gene, 0/0 genotype	28	1993-2005	11 538 (5072)	76	2270 (1138)	2005	76
Breast cancer	IGF-1 levels	17	1998-2009[b]	14 218 (4790)	71	3181 (1086)	2006	87
	DDE levels	22	1993-2001	11 544 (5222)	77	845 (456)	2000	88
	Total estradiol levels	9	1990-2000[b]	2365 (656)	74	204 (71)	1996	89
Breast cancer survival	Bone marrow micrometastasis	9	1996-2004[b]	4703 (667)	65	721 (69)	1996	90
Myocardial infarction	Lymphotoxin alpha gene, LTA exon1 10G>A	22	1998-2009	36 028 (20 640)	50	9640 (6928)	2006	91
	Adiponectin levels	7	2002-2006	4267 (1313)	70	1820 (589)	2006	70
	Hyperhomocysteinemia	21	1992-2004	19 012 (3741)	73	878 (117)	1998	92
Coronary heart disease	CRP levels[a]	48	1988-2009[b]	151 972 (10 451)	57	5457 (2009)	2004	25
Ischemic heart disease	Hyperinsulinemia	17	1979-1996	NR (1638)	59	1052 (123)	1995	93
	ACE gene, deletion-insertion polymorphism	18	1992-1997	21 876 (6573)	60	10 150 (947)	1997	94
Coronary thrombosis	Glycoprotein IIIa gene, PI^{A2} polymorphism	34	1996-2000	17 049 (8446)	66	2252 (1061)	1998	95
Vascular disease	Hyperhomocysteinemia	33	1976-1999	NR (16 097)	58	492 (123)	1995	96
Deep vein thrombosis	Hyperhomocysteinemia	27	1991-2003	9062 (3765)	61	938 (303)	2003	97
Venous thrombosis	Prothrombin gene, G20210A	79	1994-2007	49 552 (21 605)	80	5514 (2310)	2001	98
Type 2 diabetes	*TCF7L2* gene, DG10S478	36	2006-2008	74 966 (35 843)	52	6516 (3225)	2007	99
	PPARG2 gene, Pro12Ala	53	1998-2008	67 253 (28 200)	54	32 554 (14 586)	2007	100
Life-threatening bleeding with warfarin	*CYP2C9* gene, *2/*3 polymorphism	2	2000-2002	365 (NR)	67	180 (60)	2000	101
Interferon response in HCV infection	$NS5A_{2209\text{-}2248}$ protein mutations	27	1996-2003	1351 (NR)	62	84 (63)	1996	31
Pneumococcal pneumonia mortality	Penicillin resistance	9	1995-2004	3144 (436)	63	782 (108)	2002	102
Preterm delivery of low-birth-weight infant	Bacterial vaginosis	24	1990-2006	24 190 (NR)	64	2929 (493)	1995	103
Age-related macular degeneration	*CFH* gene, H1 haplotype	11	2005-2006	6816 (3679)	53	1559 (729)	2005	104
Fracture	Bone mineral density	12	1991-2004[b]	38 973 (3694)	68	163 979 (NR)	2001	37
Atrophic gastritis and intestinal metaplasia	*H pylori*	7	1995-2006	1212 (NR)	69	464 (62)	1999	105

Abbreviations: CI, confidence interval; CRP, C-reactive protein; DDE, l,l-dichloro-2,2-bis(*p*-chlorophenyl) ethylene; HCV, hepatitis C virus; *H pylori, Helicobacter pylori*; IGF-1, insulinlike growth factor 1; NR, not reported; $NS5A_{2209\text{-}2248}$, amino acid sequence 2209 to 2248 of nonstructural protein 5A.

[a] Three eligible highly cited studies exist for this topic.

[b] These meta-analyses were meta-analyses of individual patient data.

have only incremental translational value for clinical use.

The results of highly cited studies were often in stark contrast against both the largest study on the same association and the corresponding meta-analysis. Occasionally, the contrast was more prominent against the largest study. Meta-analyses of risk factors may have more inflated effects themselves, because typically they include also the highly cited studies and they may suffer from publication and other selective reporting biases.[106-111] It is probably less common to see smaller effect sizes in large studies due to poorer quality of biomarker and outcome measurements in grand-scale investigations or different population characteristics.

Several reasons could explain false-positive and inflated results among the examined highly cited investigations.[9,112] Many of these studies were relatively small and among the first to report on the association of interest. Discoveries made in small studies are prone to overestimate or underestimate the actual association.[9] Interest in

Table 3. Effect Sizes in Highly Cited Studies, Meta-analyses, and Largest Studies

Disease or Outcome	Risk Factor	Relative Risk (95% CI) in Meta-analysis[a]	Relative Risk (95% CI) in Largest Study	Relative Risk (95% CI) in Highly Cited Study	Type of Estimate (Exposure Contrast)	Representation of the Original Effects in the Meta-analysis[b]
Prostate cancer	Androgen receptor gene, CAG repeats	1.19 (1.07-1.31)	1.00 (0.96-1.03)	1.23 (0.87-1.70)	OR (≤21 vs >21 repeats)	DC
	IGF-1 levels	1.21 (1.07-1.36)	1.05 (0.92-1.19)	1.80 (1.29-2.53)	OR (per SD increase)	DC
Gastric cancer	*H pylori*	2.04 (1.69-2.45)	1.31 (0.99-1.74)	2.77 (1.04-7.97)	OR (exposed vs nonexposed)	Same
	H pylori	2.04 (1.69-2.45)	1.31 (0.99-1.74)	3.60 (1.80-7.30)	OR (exposed vs nonexposed)	S+
	H pylori	2.04 (1.69-2.45)	1.31 (0.99-1.74)	6.00 (2.10-17.3)	OR (exposed vs nonexposed)	S+
	H pylori, anti-cagA antibodies	1.64 (1.21-2.24)	1.16 (0.77-1.75)	1.99 (0.95-4.19)	OR (exposed vs nonexposed)	Same
Colon cancer	*BRCA1* gene, mutation carrier	1.19 (1.02-1.38)	0.97 (0.73-1.19)	4.11 (2.36-7.15)	OR (carriers vs noncarriers)	Same
Colorectal cancer	IGF-1 levels	1.07 (1.01-1.14)	1.04 (0.96-1.14)	1.09 (0.88-1.35)	RR (per SD increase)	DC
	MTHFR gene, C677T	1.20 (1.11-1.30)	1.37 (1.09-1.69)	1.72 (0.94-3.13)	OR (CC vs TT genotypes)	DC
Bladder cancer	*GSTM1* gene, 0/0 genotype	1.50 (1.30-1.60)	1.70 (1.40-2.00)	1.70 (1.12-2.50)	OR (null vs nonnull genotype)	Same
Breast cancer	IGF-1 levels	0.77 (0.67-0.88)	0.68 (0.51-0.90)	1.18 (0.72-1.88)	OR (lowest vs highest quintile)	S–
	DDE levels	0.97 (0.87-1.09)	1.09 (0.79-1.51)	3.68 (1.01-13.5)	OR (highest vs lowest level)	DC
	Total estradiol levels	1.29 (1.15-1.44)	1.16 (0.90-1.48)	1.60 (1.19-2.16)	RR (per doubling of estradiol levels)	DC/DA
Breast cancer survival	Bone marrow micrometastasis	1.93 (1.58-2.36)	4.04 (2.73-5.85)	4.17 (2.51-6.94)	HR (present vs absent)	Same
Myocardial infarction	Lymphotoxin alpha gene, LTA exon1 10G>A	0.98 (0.93-1.03)	1.01 (0.91-1.09)	1.78 (1.39-2.27)	OR (AA vs GG+GA genotypes)	Same
Coronary heart disease	Adiponectin levels	1.18 (0.93-1.49)	1.12 (0.85-1.49)	1.54 (1.02-2.27)	OR (bottom vs top third)	DC DA
	Hyperhomocysteinemia	1.18 (1.10-1.26)	1.07 (0.98-1.17)	2.08 (1.17-3.68)	RR (per 5-μmol/L increase)	DC/DA
	CRP levels	1.42 (1.33-1.52)	1.27 (1.13-1.43)	1.80 (1.20-2.90)	RR (per SD increased ln[CRP])	DC/DA/S+
	CRP levels	1.42 (1.33-1.52)	1.27 (1.13-1.43)	1.27 (1.13-1.43)	RR (per SD increased ln[CRP])	DC/DA/S–
	CRP levels	1.42 (1.33-1.52)	1.27 (1.13-1.43)	1.90 (1.49-2.41)	RR (per SD increased ln[CRP])	DC/DA/S–
Ischemic heart disease	Hyperinsulinemia	1.18 (1.08-1.29)	1.27 (1.08-1.49)	2.31 (1.20-4.46)	RR (per 50 pmol/L fasting or 250 pmol/L nonfasting insulin)	DC
	ACE gene, deletion-insertion polymorphism	1.16 (1.08-1.25)	1.02 (0.87-1.07)	1.07 (0.96-1.19)	OR (DD vs DI+II genotypes)	Same
Coronary thrombosis	Glycoprotein IIIa gene, PI^{A2} polymorphism	1.10 (1.03-1.18)	0.93 (0.77-1.13)	2.80 (1.20-6.40)	OR (PI^{A1A2}+PI^{A2A2} vs PI^{A1A1} genotypes)	Same

(continued)

Table 3. Effect Sizes in Highly Cited Studies, Meta-analyses, and Largest Studies (continued)

Disease or Outcome	Risk Factor	Relative Risk (95% CI) in Meta-analysis[a]	Relative Risk (95% CI) in Largest Study	Relative Risk (95% CI) in Highly Cited Study	Type of Estimate (Exposure Contrast)	Representation of the Original Effects in the Meta-analysis[b]
Vascular disease	Hyperhomocysteinemia	1.58 (1.49-1.68)	1.29 (1.09-1.58)	27.7 (3.20-240.0)	OR (increased vs normal levels)	Same
Deep vein thrombosis	Hyperhomocysteinemia	1.35 (1.11-1.66)	1.21 (1.04-1.40)	1.50 (1.06-2.11)	OR (per 5-µmol/L increase)	DC
Venous thrombosis	Prothrombin gene, G20210A	3.17 (2.91-3.46)	3.45 (2.69-4.43)	2.76 (1.36-5.60)	OR (AA+GA vs GG)	Same
Type 2 diabetes	*TCF7L2* gene, DG10S478	1.38 (1.31-1.45)	1.34 (1.24-1.44)	1.52 (1.28-1.80)	OR (T vs G allele of rs12255372)[c,d]	DC
	PPARG2 gene, Pro12Ala	1.18 (1.11-1.25)	1.14 (1.08-1.20)	4.55 (1.26-20.29)	OR (C vs G allele)	DC
Life-threatening bleeding with warfarin	*CYP2C9* gene, *2/*3 polymorphism	2.26 (1.36-3.75)	2.29 (1.18-4.64)	2.39 (1.18-4.86)	RR (*2 or *3 vs *1)	Same
Interferon response in HCV infection	$NS5A_{2209-2248}$ protein mutations	5.53 (4.50-6.79)	7.94 (5.35-11.73)	7.94 (5.35-11.73)	RR (mutant vs nonmutant isolates)	DC/DA
Pneumococcal pneumonia mortality	Penicillin resistance	1.37 (1.05-1.78)	1.50 (0.91-2.47)	1.00 (0.51-1.95)	OR (PRSP vs PSSP)	Same
Preterm delivery of low-birth-weight infant	Bacterial vaginosis	2.16 (1.56-3.00)	1.28 (0.98-1.68)	1.55 (1.20-2.01)	OR (exposed vs nonexposed)	DA
Age-related macular degeneration	*CFH* gene, H1 haplotype	2.43 (2.17-2.72)	2.05 (1.75-2.36)	2.41 (2.04-2.85)	OR (Y402H heterozygotes)	DC
Fracture	Bone mineral density	1.45 (1.39-1.51)	1.54 (1.48-1.59)	1.54 (1.48-1.59)	OR (per SD decrease)	DC/DA
Atrophic gastritis and intestinal metaplasia	*H pylori*	5.00 (3.10-8.30)	3.30 (1.30-6.60)	9.00 (1.90-41.3)	RR (exposed vs nonexposed)	S+

Abbreviations: CI, confidence interval; CRP, C-reactive protein; DD, homozygous for the deletional (D) allele; DDE, l,l-dichloro-2,2-bis(*p*-chlorophenyl) ethylene; HCV, hepatitis C virus; *H pylori, Helicobacter pylori*; DI, heterozygous for the D and I alleles; IGF-1, insulinlike growth factor 1; II, homozygous for the insertional (I) allele; $NS5A_{2209-2248}$, amino acid sequence 2209 to 2248 of nonstructural protein 5A; OR, odds ratio; PRSP, penicillin-resistant *Staphylococcus pneumoniae*; PSSP, penicillin-susceptible *Staphylococcus pneumoniae*; RR, risk ratio.

[a] According to random-effect calculations—except for hyperhomocysteinemia and vascular disease, and bone mineral density and fracture, where fixed-effect calculations have been used.

[b] DC indicates different contrast of exposure between the highly cited study and the corresponding meta-analysis; DA indicates different adjustments for covariates between the highly cited study and the corresponding meta-analysis; S+ indicates the highly cited study was represented in the corresponding meta-analysis by another study with larger sample size; and S– indicates the highly cited study was represented in the corresponding meta-analysis by another study with smaller sample size.

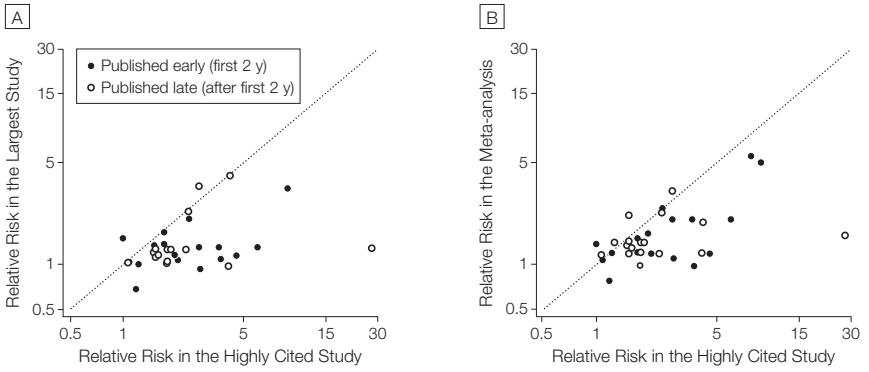
[c] The meta-analysis examined the association between type 2 diabetes and rs12255372, which is in high linkage disequilibrium (r^2=0.95) with the marker DG10S478 reported in the highly cited study.

[d] This is the summary OR for the 3 populations of the highly cited study, as they are reported in the meta-analysis.

publishing major discoveries leads to selective reporting from chasing significance. Half of the highly cited studies were published early and this may also have given them a citation advantage (more time to accrue citations). However, half of the highly cited studies were not published early in the accumulation of evidence, and often these attractive articles were among the late-appearing studies on the question of interest. There are some cases where the highly cited studies were even published after the largest study in the field. More extreme estimates of associations can be seen in both early studies and late-appearing studies.

The potential exaggeration of effects was seen in almost all cases that we analyzed, but some exceptions may

Figure. Relative Risks in the Highly Cited Studies vs the Corresponding Largest Studies and in the Highly Cited Studies vs the Corresponding Meta-analyses



Diagonal lines represent equal effects between the highly cited study and the largest study (A) or the meta-analysis (B), respectively. A, Not shown are 3 topics whereby the highly cited study was the same as the largest study. B, Meta-analyses may include the data from the highly cited studies, but the latter are usually small compared with the corresponding meta-analyses (median, 5%; interquartile range, 2%-12%, of the meta-analysis sample size).

also occur. Even in those uncommon exceptions where the effect estimate was smaller in the highly cited study than in the meta-analysis, a similar mechanism of preference for attention-drawing results may have applied, but simply a "negative" result was felt to carry more notoriety. For example, in 1 occasion the highly cited study showed that penicillin resistance unexpectedly did not increase the risk of death from pneumococcal pneumonia,[32] while the meta-analysis showed an increase in mortality.[63] However, in the large majority, notoriety is associated with large rather than paradoxical null effects.

We should acknowledge that several of the highly cited biomarkers probably have genuine associations; a minority have even large effects, eg, the associations of gene loci such as *CFH* with age-related macular degeneration,[18] prothrombin *G2010A* and venous thrombosis,[49] and *CYP2C9* and bleeding risk[36]; the associations of *H pylori* with gastric cancer and atrophic gastritis[21-23,38]; or the association of NS5A mutations with interferon response.[31] Nevertheless, even in these cases, highly cited studies described often optimistic effect estimates. Some of these biomarkers have started being used in clinical practice.[113] Their cost-utility depends on their cost and on their discriminating ability.[114,115] If discriminating ability is overestimated, markers may become overpriced. Biomarkers with large populations of potential users could cause major escalation of health care costs with limited benefits. Obviously, adopting markers with no discriminating ability would be even worse. Clinical biomarker use requires robust evidence and safeguards.[3,116,117]

Novel biomarkers that purport to assist in complex problems related to decisions about diagnosis and prognosis with relatively simple objective measures are often viewed oversimplistically as major advances. However, biomarkers may start having a potential clinical utility when it can be demonstrated that they can provide incremental information beyond already known predictors and any other known biomarkers.[118] Regardless of such adjustments, the results of highly cited biomarker studies that appear in major journals are often substantially overestimated. This should lead to reinforcing healthy skepticism about interpreting this literature. This does not mean that no biomarkers of any use are possible to discover, but that the standards for claiming success should be higher. These standards should include not only prospective design, careful analysis plans, and meticulous reporting, but also extensive replication and validation of proposed biomarkers in large independent studies and assessment of their incremental ability. Until such studies are available, emphasis on single studies with highly promising results may be premature.

Some limitations should be discussed. First, we selected only highly cited studies that presented RRs in their abstracts. This removed subjectivity in selecting the most important RR estimate among the many presented in the full text of these articles. Moreover, many highly cited biomarker studies do not report RR estimates in their abstract and were thus excluded from our sample. It is unlikely, however, that the replication record of such biomarkers would be better. Second, we were able to retrieve meta-analyses corresponding to only a third of the highly cited primary studies. It is unlikely that biomarkers lacking a corresponding meta-analysis are enriched in successful replications. If anything, meta-analyses are more likely to conduct for biomarkers that had more and successful replications. Third, the results may not be directly extrapolated to studies of biomarkers that do not get much cited. Citation bias, the preference to cite extreme results, is described in diverse fields.[119-122] However, less-cited biomarkers are probably less influential in scientific circles and less likely to draw enough attention to become applied in practice. Finally, there is no consensus on what threshold characterizes a highly cited study. One can set also citation criteria that adjust for the time since publication to compare citations of articles published in very different years. However, we preferred to select articles that have clearly achieved cumulative recognition in the literature rather than those that may (or may not) achieve this in the future.

Merely because an article is highly cited does not indicate that the reason for citing it is that it is considered the best science. It is difficult to judge the quality of biomarker studies, mostly because reporting of methods in this literature has been largely elliptical to date.[11,13,123] This further reinforces the notion that citing investigators pay more attention to reporting more extreme estimates rather than the robustness of the methods, which is often either intangible or difficult to compare between different studies. Readers should be cautious when authors cite single studies and not meta-analyses, and authors should be more careful in what they cite.

While we acknowledge these caveats, our study documents that results in highly cited biomarker studies often significantly overestimate the findings seen from meta-analyses. Evidence from multiple studies, in particular large investigations, is necessary to appreciate the discriminating ability of these emerging risk factors. Rapid clinical adoption in the absence of such evidence may lead to wasted resources.

Author Contributions: Dr Ioannidis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Ioannidis.
Acquisition of data: Ioannidis, Panagiotou.
Analysis and interpretation of data: Ioannidis, Panagiotou.
Drafting of the manuscript: Ioannidis.
Critical revision of the manuscript for important intellectual content: Ioannidis, Panagiotou.
Statistical analysis: Ioannidis, Panagiotou.
Study supervision: Ioannidis.
Conflict of Interest Disclosures: Both authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.
Online-Only Materials: The eTable and eFigures 1 and 2 are available at http://www.jama.com.

REFERENCES

1. Moons KG, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research. *BMJ*. 2009;338:b375.
2. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research. *BMJ*. 2009; 338:b606.

3. Hlatky MA, Greenland P, Arnett DK, et al; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk. *Circulation*. 2009; 119(17):2408-2416.
4. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. *Eur J Cancer*. 2007;43(17): 2559-2579.
5. Kavvoura FK, Liberopoulos G, Ioannidis JP. Selection in reported epidemiological risks. *PLoS Med*. 2007; 4(3):e79.
6. Tzoulaki I, Liberopoulos G, Ioannidis JP. Assessment of claims of improved prediction beyond the Framingham risk score. *JAMA*. 2009;302(21):2345-2352.
7. Riley RD, Burchill SA, Abrams KR, et al. A systematic review and evaluation of the use of tumour markers in paediatric oncology. *Health Technol Assess*. 2003; 7(5):1-162.
8. Vergouwe Y, Steyerberg EW, Eijkemans MJ, Habbema JD. Validity of prognostic models. *Semin Urol Oncol*. 2002; 20(2):96-107.
9. Ioannidis JP. Why most discovered true associations are inflated. *Epidemiology*. 2008;19(5):640-648.
10. Altman DG. Systematic reviews of evaluations of prognostic variables. *BMJ*. 2001;323(7306):224-228.
11. Kyzas PA, Loizou KT, Ioannidis JP. Selective reporting biases in cancer prognostic factor studies. *J Natl Cancer Inst*. 2005;97(14):1043-1055.
12. Hayden JA, Côté P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med*. 2006;144(6):427-437.
13. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Quality of reporting of cancer prognostic marker studies. *J Natl Cancer Inst*. 2007;99(3):236-243.
14. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177-188.
15. Ozaki K, Ohnishi Y, Iida A, et al. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. *Nat Genet*. 2002; 32(4):650-654.
16. Giovannucci E, Stampfer MJ, Krithivas K, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc Natl Acad Sci U S A*. 1997;94(7):3320-3323.
17. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet*. 2006;38 (3):320-323.
18. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2005;102(20):7227-7232.
19. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet*. 1998;20 (3):284-287.
20. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk. *Science*. 1998;279(5350):563-566.
21. Forman D, Newell DG, Fullerton F, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer. *BMJ*. 1991;302(6788):1302-1305.
22. Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med*. 1991;325(16):1127-1131.
23. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med*. 1991;325(16):1132-1136.
24. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342(12):836-843.
25. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350(14):1387-1397.
26. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ*. 2000; 321(7255):199-204.
27. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia. *N Engl J Med*. 1991;324(17):1149-1155.
28. Després JP, Lamarche B, Mauriège P, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med*. 1996;334 (15):952-957.
29. Lindpaintner K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med*. 1995;332(11):706-711.
30. den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med*. 1996;334(12):759-762.
31. Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med*. 1996;334(2): 77-81.
32. Pallares R, Liñares J, Vadillo M, et al. Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain. *N Engl J Med*. 1995;333(8):474-480.
33. Hillier SL, Nugent RP, Eschenbach DA, et al; The Vaginal Infections and Prematurity Study Group. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med*. 1995; 333(26):1737-1742.
34. Braun S, Pantel K, Müller P, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med*. 2000;342(8):525-533.
35. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. *N Engl J Med*. 1996;334(17):1090-1094.
36. Higashi MK, Veenstra DL, Kondo LM, et al. Association between *CYP2C9* genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA*. 2002;287(13):1690-1698.
37. Siris ES, Miller PD, Barrett-Connor E, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women. *JAMA*. 2001;286(22):2815-2822.
38. Kuipers EJ, Uyterlinde AM, Peña AS, et al. Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet*. 1995;345(8964):1525-1528.
39. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004;291(14): 1730-1737.
40. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet*. 1998;351 (9113):1393-1396.
41. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE; Breast Cancer Linkage Consortium. Risks of cancer in *BRCA1*-mutation carriers. *Lancet*. 1994; 343(8899):692-695.
42. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA*. 1992; 268(7):877-881.
43. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *J Natl Cancer Inst*. 1995;87(3):190-197.
44. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res*. 1997;57(6):1098-1102.
45. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. *J Natl Cancer Inst*. 1993;85(14):1159-1164.
46. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst*. 1993;85(8):648-652.
47. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst*. 1999;91 (7):620-625.
48. Blaser MJ, Perez-Perez GI, Kleanthous H, et al. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*. 1995; 55(10):2111-2115.
49. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88(10):3698-3703.
50. Li W, Xu J, Wang X, et al. Lack of association between lymphotoxin-alpha, galectin-2 polymorphisms and coronary artery disease. *Atherosclerosis*. 2010;208(2):433-436.
51. Zeegers MP, Kiemeney LA, Nieder AM, Ostrer H. How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? *Cancer Epidemiol Biomarkers Prev*. 2004;13(11 pt 1):1765-1771.
52. Tong Y, Lin Y, Zhang Y, et al. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus. *BMC Med Genet*. 2009; 10:15.
53. Conley YP, Jakobsdottir J, Mah T, et al. *CFH*, *ELOVL4*, *PLEKHA1* and *LOC387715* genes and susceptibility to age-related maculopathy. *Hum Mol Genet*. 2006;15(21):3206-3218.
54. Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus. *Am J Epidemiol*. 2010;171(6):645-655.
55. Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, Martin RM. Circulating insulin-like growth factor peptides and prostate cancer risk. *Int J Cancer*. 2009; 124(10):2416-2429.
56. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of *Helicobacter pylori* infection with gastric carcinoma. *Am J Gastroenterol*. 1999;94(9):2373-2379.
57. Kaptoge S, Di Angelantonio E, Lowe G, et al; Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality. *Lancet*. 2010;375(9709): 132-140.
58. Cleophas TJ, Hornstra N, van Hoogstraten B, van der Meulen J. Homocysteine, a risk factor for coronary artery disease or not? *Am J Cardiol*. 2000; 86(9):1005-1009.
59. Ruige JB, Assendelft WJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM. Insulin and risk of cardiovascular disease. *Circulation*. 1998;97(10):996-1001.
60. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. *ACE* gene polymorphism in cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2000;20(2):484-492.
61. Den Heijer M, Lewington S, Clarke R. Homocys-

teine, MTHFR and risk of venous thrombosis. *J Thromb Haemost*. 2005;3(2):292-299.
62. Schinkel J, Spaan WJ, Kroes AC. Meta-analysis of mutations in the NS5A gene and hepatitis C virus resistance to interferon therapy. *Antivir Ther*. 2004; 9(2):275-286.
63. Tleyjeh IM, Tlaygeh HM, Hejal R, Montori VM, Baddour LM. The impact of penicillin resistance on short-term mortality in hospitalized adults with pneumococcal pneumonia. *Clin Infect Dis*. 2006;42 (6):788-797.
64. Leitich H, Kiss H. Asymptomatic bacterial vaginosis and intermediate flora as risk factors for adverse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol*. 2007;21(3):375-390.
65. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med*. 2005;353(8):793-802.
66. Di Castelnuovo A, de Gaetano G, Donati MB, Iacoviello L. Platelet glycoprotein receptor IIIa polymorphism PLA1/PLA2 and coronary risk. *Thromb Haemost*. 2001;85(4):626-633.
67. Sanderson S, Emery J, Higgins J. *CYP2C9* gene variants, drug dose, and bleeding risk in warfarin-treated patients. *Genet Med*. 2005;7(2):97-104.
68. Johnell O, Kanis JA, Oden A, et al. Predictive value of BMD for hip and other fractures. *J Bone Miner Res*. 2005;20(7):1185-1194.
69. Adamu MA, Weck MN, Gao L, Brenner H. Incidence of chronic atrophic gastritis. *Eur J Epidemiol*. 2010;25(7):439-448.
70. Sattar N, Wannamethee G, Sarwar N, et al. Adiponectin and coronary heart disease. *Circulation*. 2006; 114(7):623-629.
71. Key TJ, Appleby PN, Reeves GK, Roddam AW; Endogenous Hormones and Breast Cancer Collaborative Group. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk. *Lancet Oncol*. 2010;11(6):530-542.
72. Friedenson B. *BRCA1* and *BRCA2* pathways and the risk of cancers other than breast or ovarian. *MedGenMed*. 2005;7(2):60.
73. Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M. Homocysteine level and coronary heart disease incidence. *Mayo Clin Proc*. 2008;83(11):1203-1212.
74. Key T, Appleby P, Barnes I, Reeves G; Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women. *J Natl Cancer Inst*. 2002; 94(8):606-616.
75. Taioli E, Garza MA, Ahn YO, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer. *Am J Epidemiol*. 2009;170(10):1207-1221.
76. García-Closas M, Malats N, Silverman D, et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer. *Lancet*. 2005;366(9486):649-659.
77. López-Cervantes M, Torres-Sánchez L, Tobías A, López-Carrillo L. Dichlorodiphenyldichloroethane burden and breast cancer risk. *Environ Health Perspect*. 2004;112(2):207-214.
78. Rinaldi S, Cleveland R, Norat T, et al. Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk. *Int J Cancer*. 2010;126(7):1702-1715.
79. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology*. 2003;125(6):1636-1644.
80. Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. *Thromb Haemost*. 2009; 102(2):360-370.
81. Edwards SM, Badzioch MD, Minter R, et al. Androgen receptor polymorphisms. *Int J Cancer*. 1999; 84(5):458-465.
82. Severi G, Morris HA, MacInnis RJ, et al. Circulating insulin-like growth factor-I and binding protein-3 and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2006;15(6):1137-1141.
83. Sipponen P, Riihelä M, Hyvärinen H, Seppälä K. Chronic nonatropic ("superficial") gastritis increases the risk of gastric carcinoma. *Scand J Gastroenterol*. 1994;29(4):336-340.
84. Wu AH, Crabtree JE, Bernstein L, et al. Role of *Helicobacter pylori* CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer*. 2003;103(6):815-821.
85. Evans HS, Lewis CM, Robinson D, Bell CM, Møller H, Hodgson SV. Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. *Br J Cancer*. 2001;84 (3):435-440.
86. Ulvik A, Vollset SE, Hansen S, Gislefoss R, Jellum E, Ueland PM. Colorectal cancer and the methylenetetrahydrofolate reductase 677C->T and methionine synthase 2756A->G polymorphisms. *Cancer Epidemiol Biomarkers Prev*. 2004;13(12):2175-2180.
87. Rinaldi S, Peeters PH, Berrino F, et al. IGF-I, IGFBP-3 and breast cancer risk in women. *Endocr Relat Cancer*. 2006;13(2):593-605.
88. Millikan R, DeVoto E, Duell EJ, et al. Dichlorodiphenyldichloroethene, polychlorinated biphenyls, and breast cancer among African-American and white women in North Carolina. *Cancer Epidemiol Biomarkers Prev*. 2000;9(11):1233-1240.
89. Dorgan JF, Longcope C, Stephenson HE Jr, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. *Cancer Epidemiol Biomarkers Prev*. 1996;5(7):533-539.
90. Diel IJ, Kaufmann M, Costa SD, et al. Micrometastatic breast cancer cells in bone marrow at primary surgery. *J Natl Cancer Inst*. 1996;88(22):1652-1658.
91. Clarke R, Xu P, Bennett D, et al; International Study of Infarct Survival (ISIS) Collaborators. Lymphotoxin-alpha gene and risk of myocardial infarction in 6,928 cases and 2,712 controls in the ISIS case-control study. *PLoS Genet*. 2006;2(7):e107.
92. Stehouwer CD, Weijenberg MP, van den Berg M, Jakobs C, Feskens EJ, Kromhout D. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men. *Arterioscler Thromb Vasc Biol*. 1998;18(12):1895-1901.
93. Møller LF, Jespersen J. Fasting serum insulin levels and coronary heart disease in a Danish cohort. *J Cardiovasc Risk*. 1995;2(3):235-240.
94. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sørensen TI, Jensen G, Tybjaerg-Hansen A. ACE gene polymorphism: ischemic heart disease and longevity in 10,150 individuals. *Circulation*. 1997;95(10): 2358-2367.
95. Gardemann A, Humme J, Stricker J, et al. Association of the platelet glycoprotein IIIa PlA1/A2 gene polymorphism to coronary artery disease but not to nonfatal myocardial infarction in low risk patients. *Thromb Haemost*. 1998;80(2):214-217.
96. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol*. 1995;24 (4):704-709.
97. Tsai AW, Cushman M, Tsai MY, et al. Serum homocysteine, thermolabile variant of methylene tetrahydrofolate reductase (MTHFR), and venous thromboembolism. *Am J Hematol*. 2003;72(3): 192-200.
98. Emmerich J, Rosendaal FR, Cattaneo M, et al; Study Group for Pooled-Analysis in Venous Thromboembolism. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism. *Thromb Haemost*. 2001;86(3):809-816.
99. Kimber CH, Doney AS, Pearson ER, et al. TCF7L2 in the Go-DARTS study. *Diabetologia*. 2007;50 (6):1186-1191.
100. Zeggini E, Weedon MN, Lindgren CM, et al; Wellcome Trust Case Control Consortium (WTCCC). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*. 2007;316(5829):1336-1341.
101. Margaglione M, Colaizzo D, D'Andrea G, et al. Genetic modulation of oral anticoagulation with warfarin. *Thromb Haemost*. 2000;84(5):775-778.
102. Pallares R, Capdevila O, Liñares J, et al. The effect of cephalosporin resistance on mortality in adult patients with nonmeningeal systemic pneumococcal infections. *Am J Med*. 2002;113(2):120-126.
103. Meis PJ, Goldenberg RL, Mercer B, et al; National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. The preterm prediction study. *Am J Obstet Gynecol*. 1995; 173(4):1231-1235.
104. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14(21):3227-3236.
105. Ozasa K, Kurata JH, Higashi A, et al. *Helicobacter pylori* infection and atrophic gastritis. *Dig Dis Sci*. 1999;44(2):253-256.
106. Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet*. 1991; 337(8746):867-872.
107. Dwan K, Altman DG, Arnaiz JA, et al. Systematic review of the empirical evidence of study publication bias and outcome reporting bias. *PLoS One*. 2008;3(8):e3081.
108. Egger M, Schneider M, Davey Smith G. Spurious precision? *BMJ*. 1998;316(7125):140-144.
109. Thompson S, Ekelund U, Jebb S, et al. A proposed method of bias adjustment for meta-analyses of published observational studies [published online April 21, 2011]. *Int J Epidemiol*. doi:10.1093/ije/dyq248.
110. Greenland S, Copas J, Jones DR, et al. Multiple-bias modelling for analysis of observational data. *J R Stat Soc Ser A Stat Soc*. 2005;168(2):267-306.
111. Salanti G, Ioannidis JP. Synthesis of observational studies should consider credibility ceilings. *J Clin Epidemiol*. 2009;62(2):115-122.
112. Ioannidis JP. Why most published research findings are false. *PLoS Med*. 2005;2(8):e124.
113. Fock KM, Katelaris P, Sugano K, et al; Second Asia-Pacific Conference. Second Asia-Pacific Consensus Guidelines for *Helicobacter pylori* infection. *J Gastroenterol Hepatol*. 2009;24(10):1587-1600.
114. Ioannidis JP. Personalized genetic prediction. *Ann Intern Med*. 2009;150(2):139-141.
115. Eckman MH, Rosand J, Greenberg SM, Gage BF. Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation. *Ann Intern Med*. 2009; 150(2):73-83.
116. Vasan RS. Biomarkers of cardiovascular disease. *Circulation*. 2006;113(19):2335-2362.
117. Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease. *JAMA*. 2003;290 (7):932-940.
118. Ioannidis JP, Tzoulaki I. What makes a good predictor? *JAMA*. 2010;303(16):1646-1647.
119. Greenberg SA. How citation distortions create unfounded authority. *BMJ*. 2009;339:b2680.
120. Kjaergard LL, Gluud C. Citation bias of hepatobiliary randomized clinical trials. *J Clin Epidemiol*. 2002; 55(4):407-410.
121. Ross D, Whitehead M, Stevenson J. Use of hormone replacement therapy. *BMJ*. 1996;313(7058): 686-687.
122. Ravnskov U. Cholesterol lowering trials in coronary heart disease. *BMJ*. 1992;305(6844):15-19.
123. Rifai N, Altman DG, Bossuyt PM. Reporting bias in diagnostic and prognostic studies. *Clin Chem*. 2008; 54(7):1101-1103.