Predizione genetica del rischio cardiovascolare

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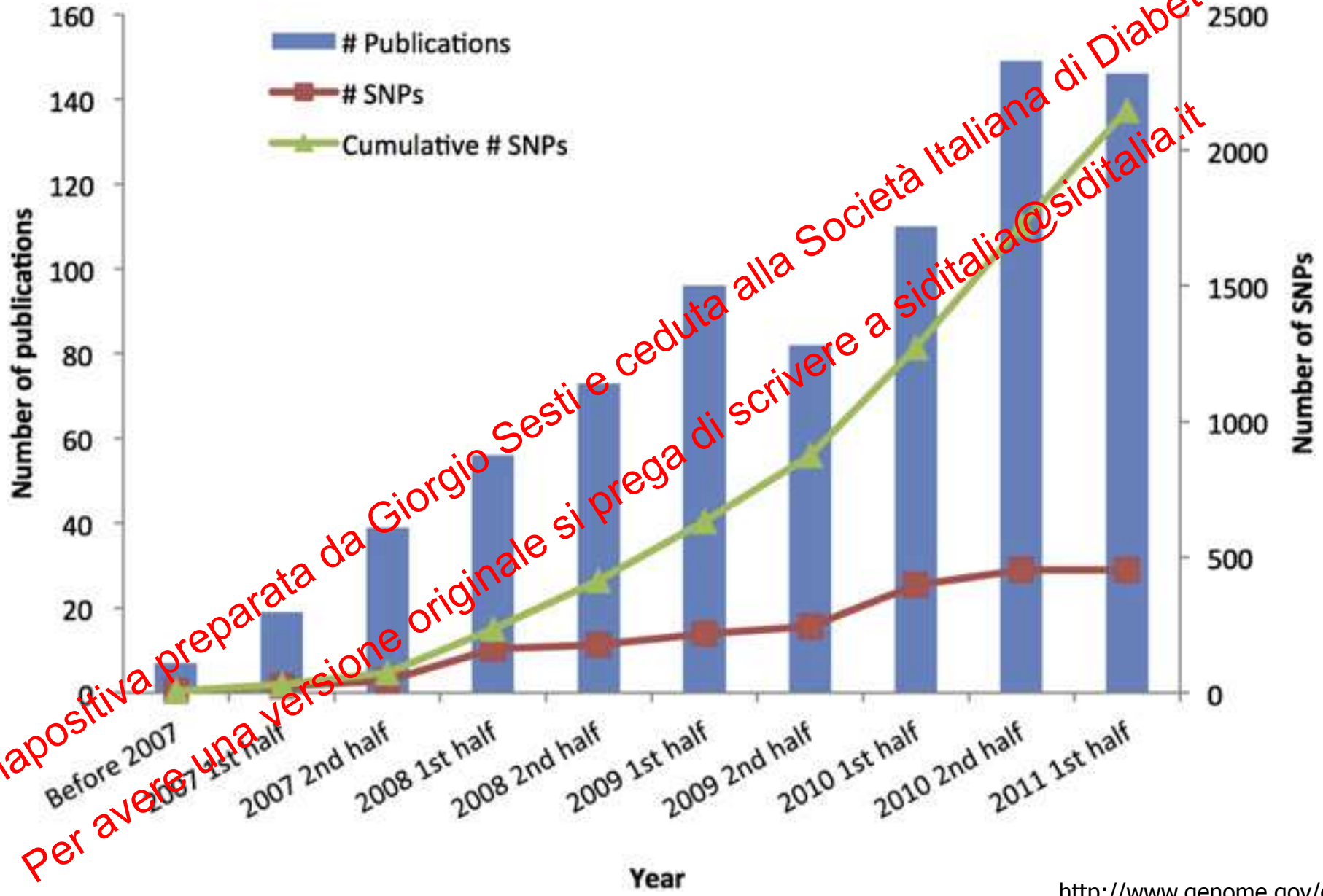
CVD has genetic and environmental determinants, and is the leading cause of morbidity and mortality worldwide.

It is estimated that nearly 15% to 20% of MI patients have none of the traditional risk factors and would be considered "low risk" by current risk prediction scores.

The heritability of MI, which provides an estimate of the genetic variance in MI risk, has been estimated at 40% to 60%.

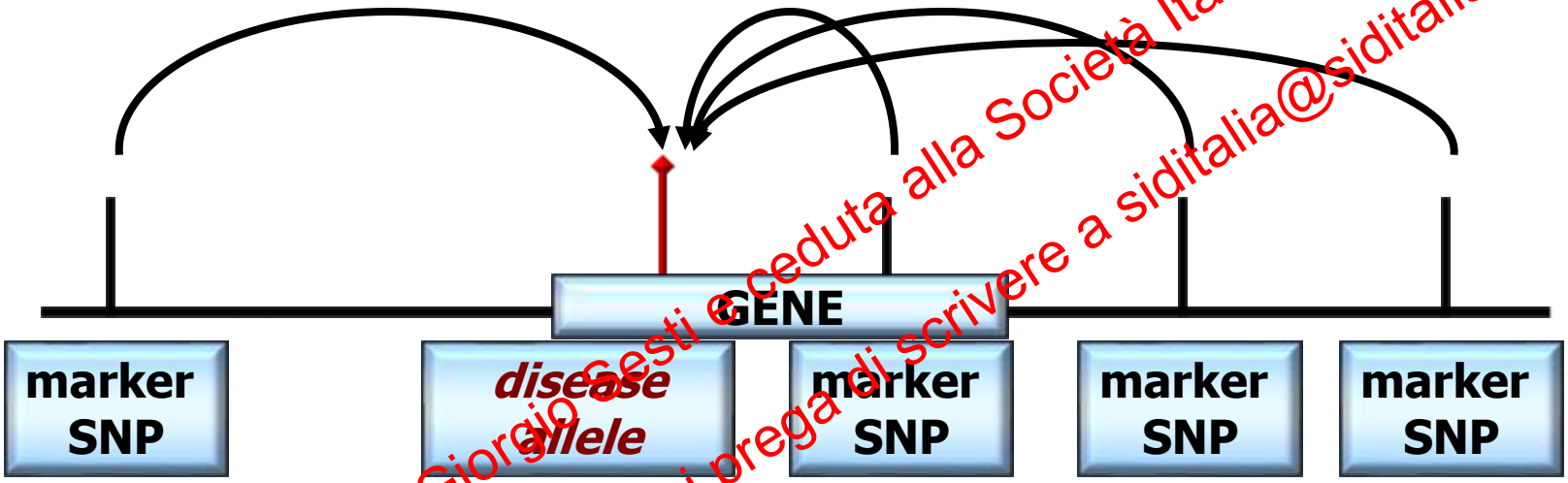
Published GWA Reports



http://www.genome.gov/gwastudies

Why genome-wide association studies?

SNP association with disease allele



Why genome-wide association studies?

Affymetrix GeneChip Human Mapping 100K array (115K SNPs)

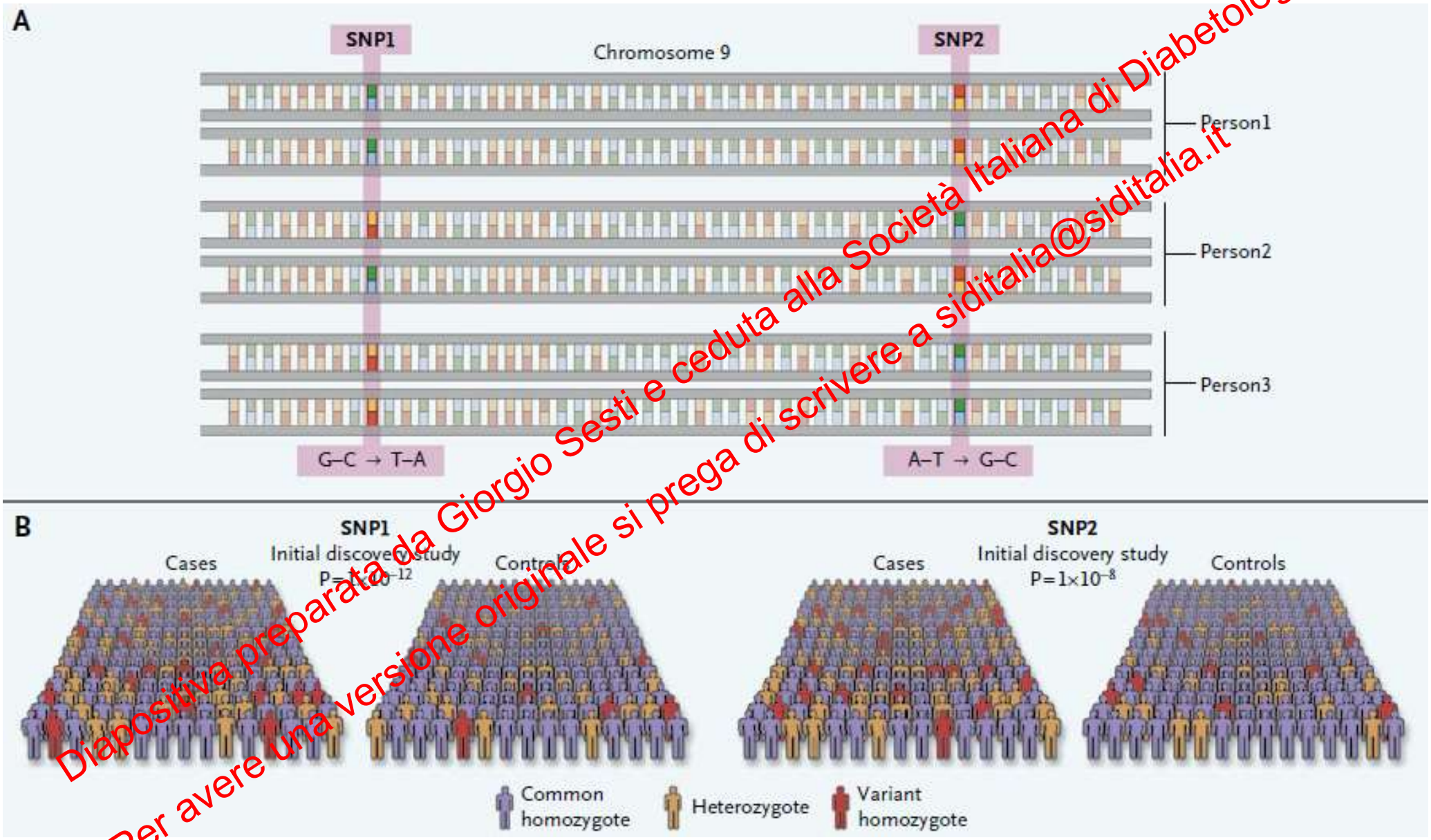
Affymetrix Genome-Wide Human SNP Array 5.0 (500K SNPs)

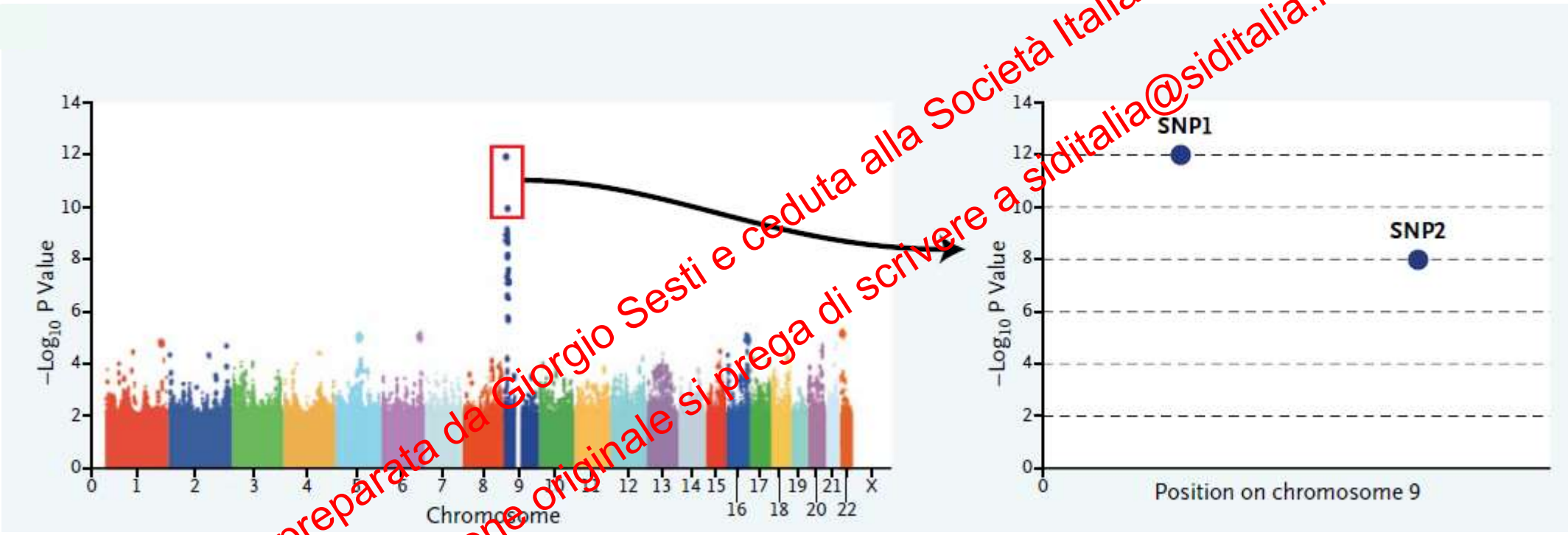
Affymetrix Genome-Wide Human SNP Array 6.0 (906K SNPs)

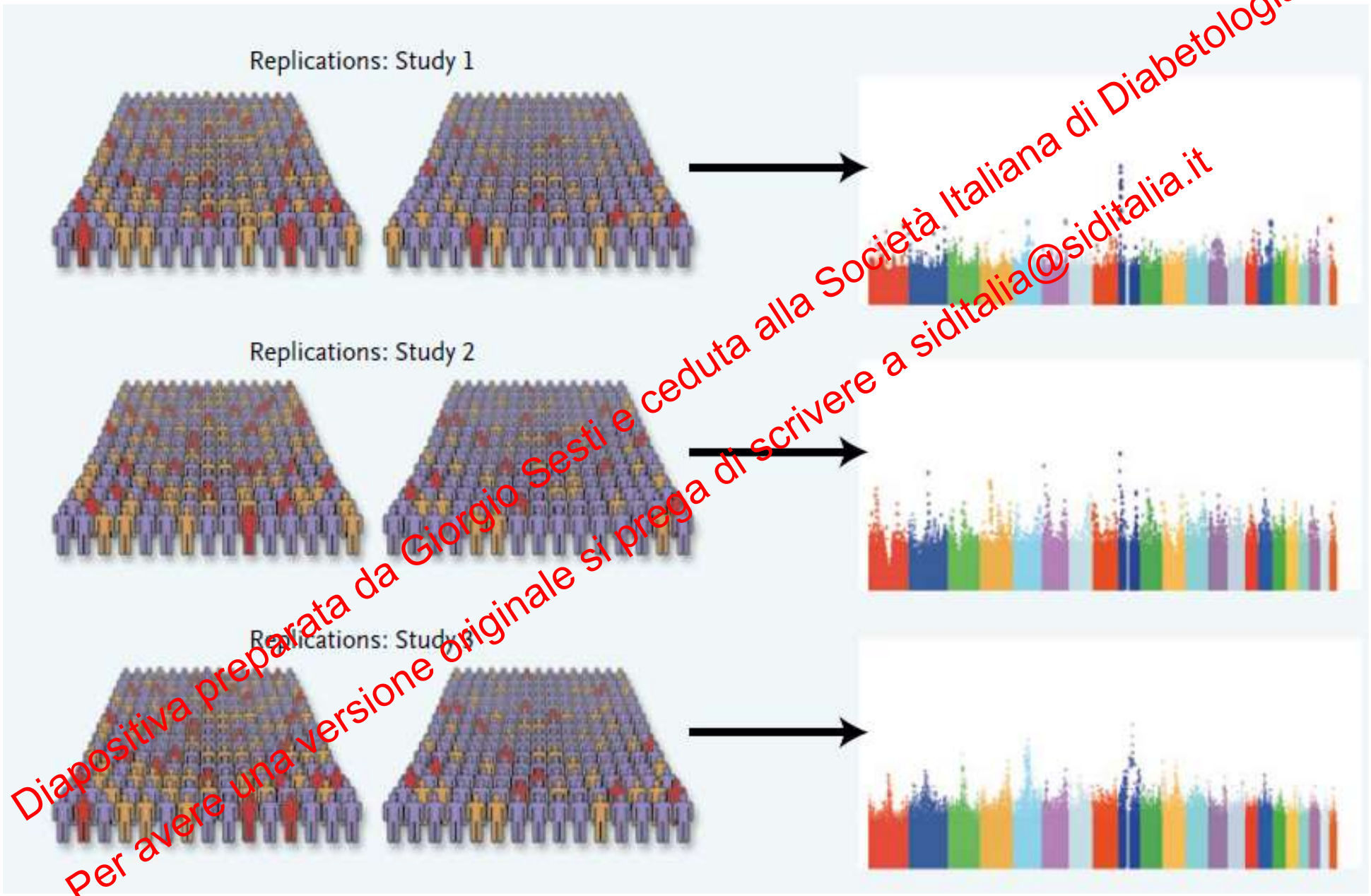
Illumina HumanHap300 Genotyping BeadChip (317K SNPs) Illumina

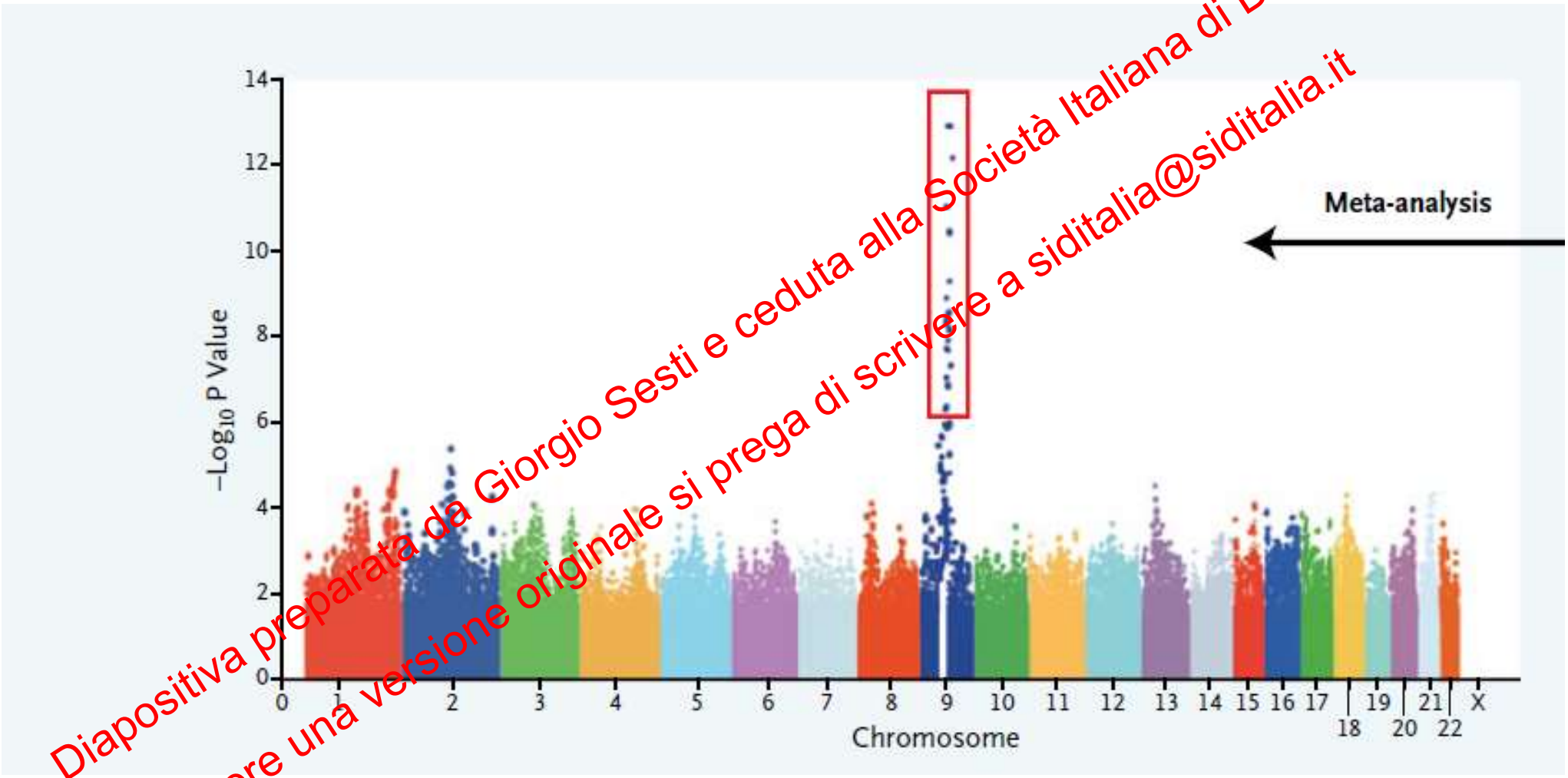
HumanHap550 Genotyping BeadChip (550K SNPs)

Illumina HumanHap Genotyping BeadChip (1M SNPs)

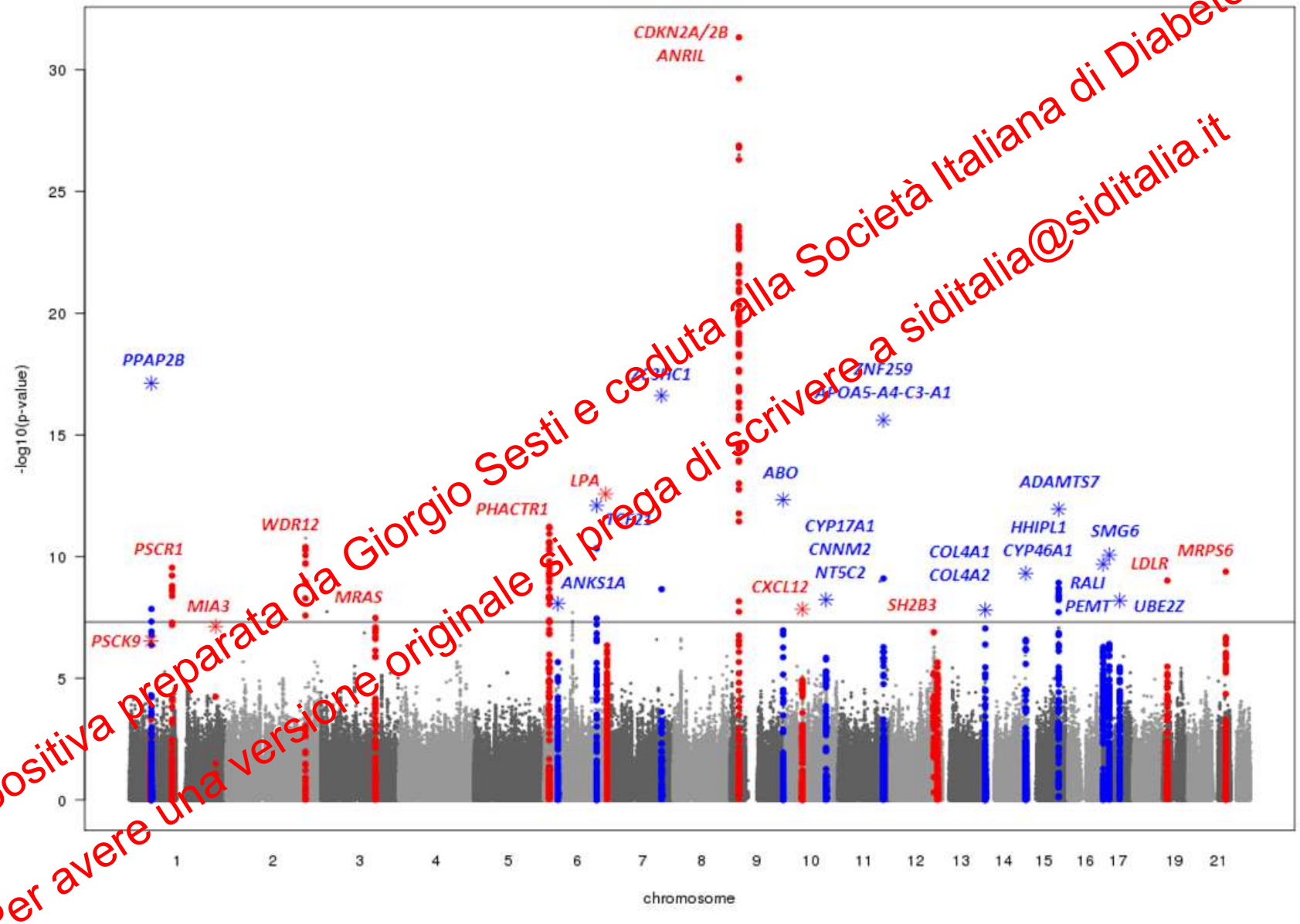






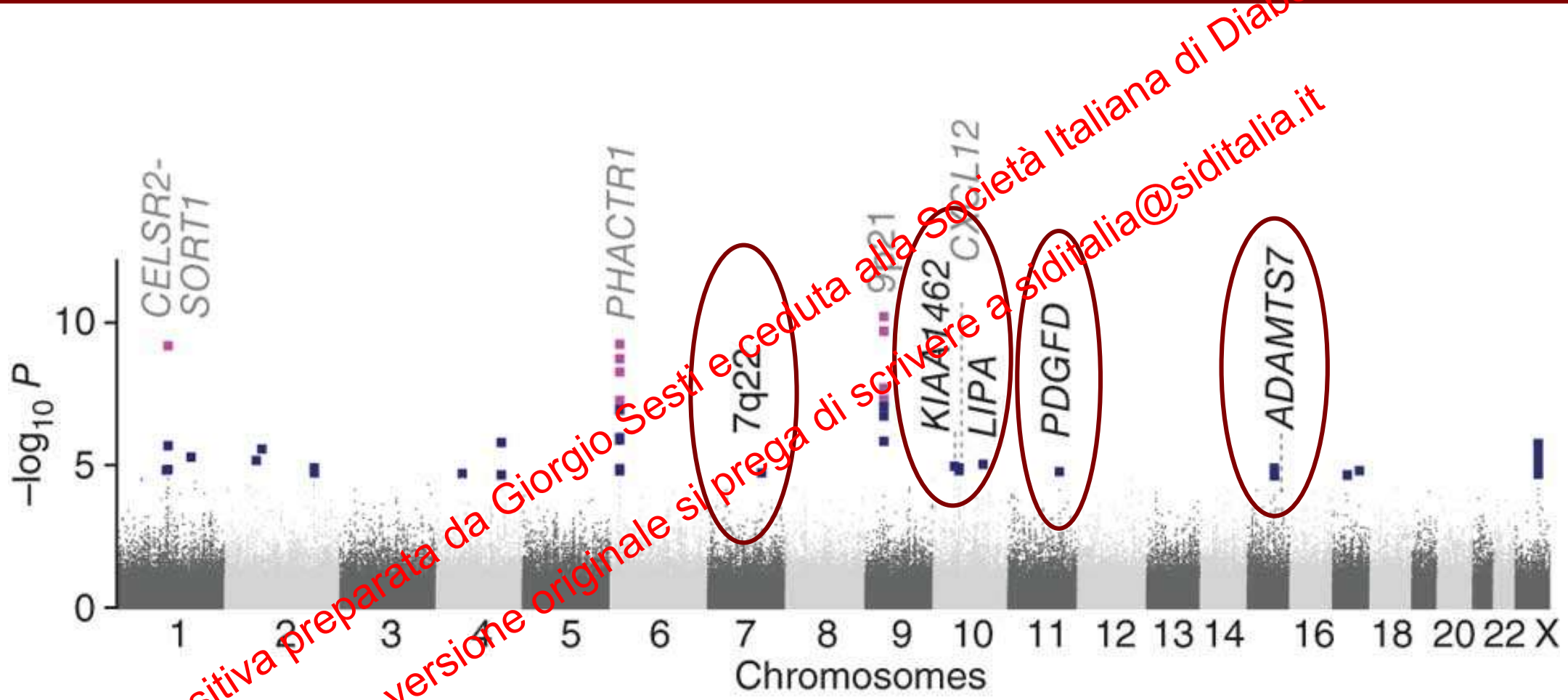


Large-scale association analyses identifies 13 new susceptibility loci for coronary artery disease



Schunkert H et al. Nat Genet 43: 333–338, 2011

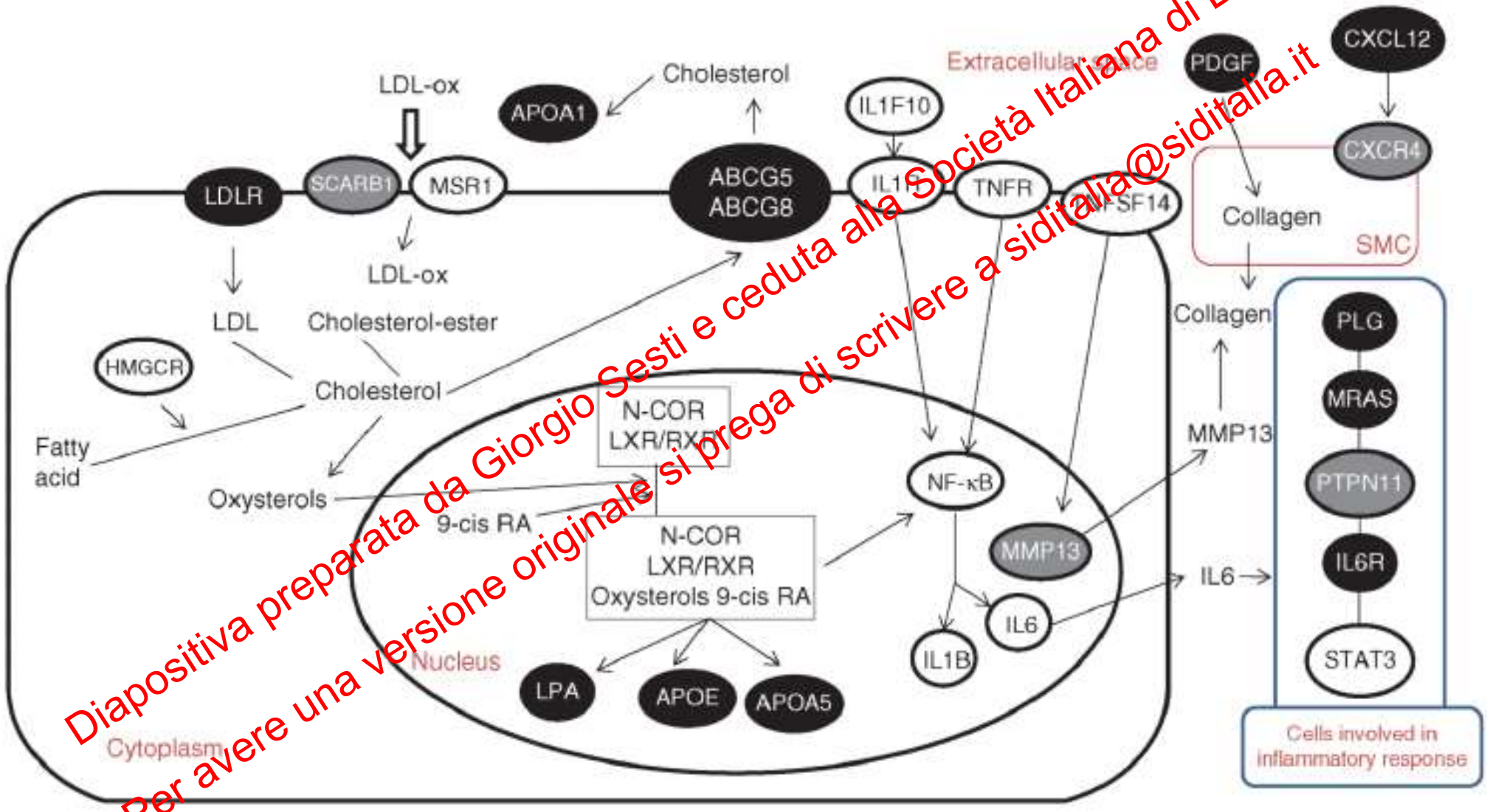
A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. The Coronary Artery Disease (C4D) Genetics Consortium



Nat Genet 43:339-44, 2011

Network analysis constructed with the top 222 SNPs

The top pathways, atherosclerosis signaling, liver X receptor (LXR)/retinoid X receptor (RXR) activation and farnesoid X receptor (FXR)/RXR activation, all harbor genes involved in lipid metabolism in addition to the acute phase response signaling (AAPRS) pathway.



CARDIoGRAMplusC4D Consortium . Nat Genet 45: 25–33, 2013

Clinical application of diabetes genetic information:

Can genetic markers improve CVD risk prediction over and above validated risk algorithms such as the Framingham risk score and a family history of CVD?

Attributes of risk factors used in disease prediction

Property	Nongenetic risk factors	Genetic risk factors
Cost	Can be high*	Low and falling
Setting of test	Can require on-site attendance*	Genotyping and sequencing can be conducted remotely using a dispatched DNA sample
Test duration	Can be lengthy*	Rapid
Measurement error (reliability)	Can be operator dependent*	High fidelity
Variability	Can alter with age*, fluctuate in the short term‡, and be modified by subclinical disease	Stable throughout lifetime, not modified by disease

*For example, when using imaging-based methods to measure carotid intima-media thickness. ‡For example, for blood biomarkers such as C-reactive protein.

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

Common features of CAD genetic risk variants discovered by GWAS

- **GWAS studies have identified variants that mediate their risk through lipids or through hypertension and loci acting independently of known risk factors.**

Risk loci associated with cholesterol or hypertension discovered by GWAS for CHD/MI

Chromosomal Location	SNP	Nearby Genes	Risk Allele Frequency (Allele)	Odds Ratio (95% CI)
Loci that associate with cholesterol				
1p32.3	rs11206510	*PCSK9*	0.82 (T)	1.15 (1.10–1.21)
1p13.3	rs599839	*SORT1*	0.78 (A)	1.29 (1.18–1.40)
2p21	Rs4299376	*ABCG8*	0.33 (G)	1.08 (1.04–1.13)
6q25.3	rs3798220	*LPA*	0.02 (C)	1.92 (1.48–2.49)
8q24.13	rs17321515	*TRIB1*	0.54 (A)	1.10 (1.06–1.15)
9q34.2*	rs579459	*ABO*	0.21 (C)	1.10 (1.07–1.13)
11q23.3	rs964184	*ZNF259, APOA5-A4-C3-A1*	0.13 (G)	1.13 (1.10–1.16)
19p13.2	rs1122608	*LDLR*	0.77 (G)	1.14 (1.09–1.19)
Loci that associate with hypertension				
10q24.32	rs12413409	*CYP17A1, CNNM2, NT5C2*	0.89 (G)	1.12 (1.08–1.16)
12q24.12	rs3184504	*SH2B3*	0.44 (T)	1.13 (1.08–1.18)

Roberts R et al. J Am Coll Cardiol 60:1715–21, 2012

Common features of CAD genetic risk variants discovered by GWAS

- **Loci underpinning LDL-C levels account for only 12–15% of the variance of this quantitative trait.**

- **The increased relative risk (odds ratio) of each variant for CVD is small with a mean increased risk of 18%.**

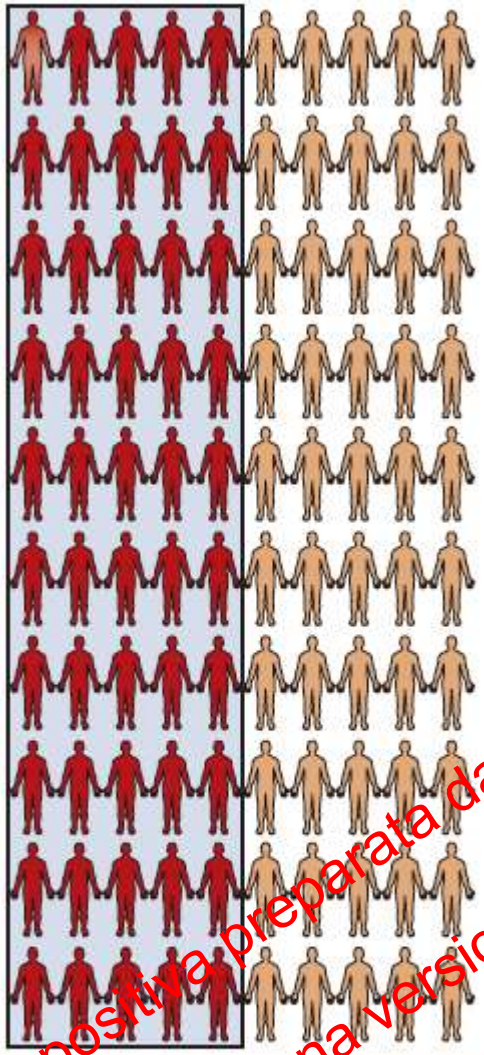
Risk loci that do not associate with known risk factors discovered by GWAS for CHD/MI

Chromosomal Location	SNP	Nearby Genes	Risk Allele Frequency (Allele)	Odds Ratio (95% CI)
Loci that do not associate with known risk factors				
1p32.2	rs17114036	*PPAP2B*	0.91 (A)	1.17 (1.13–1.22)
1q41	rs17465637	*MIA3*	0.74 (C)	1.20 (1.12–1.30)
2q33.1	rs6725887	*WDR12*	0.15 (C)	1.16 (1.10–1.22)
3q22.3	rs2306374	*MRAS*	0.18 (C)	1.15 (1.11–1.19)
5q31.1	Rs2706399	*IL5*	0.52 (G)	1.07 (1.03–1.11)
6p24.1	rs6903956	*C6orf105*	0.07 (A)	1.65 (1.44–1.90)
6p24.1	rs12526453	*PHACTR1*	0.61 (C)	1.13 (1.09–1.17)
6p21.31	rs17609940	*ANKS1A*	0.75 (G)	1.07 (1.05–1.10)
6q23.2	rs12190287	*TCF21*	0.62 (C)	1.08 (1.06–1.10)
7q22.3	rs10953541	*BCAP29*	0.75 (C)	1.08 (1.05–1.11)
7q32.2	rs11556924	*ZC3HC1*	0.62 (C)	1.09 (1.07–1.12)
9p21.3	rs4977574	*CDKN2A, CDKN2B, ANRIL*	0.46 (G)	1.25 (1.18–1.31) 1.37 (1.26–1.48)
10p11.23	rs2505083	*KIAA1462*	0.42 (C)	1.07 (1.04–1.09)
10q11.21	rs1746048	*CXCL12*	0.87 (C)	1.33 (1.20–1.48)
10q23.31	rs1412444	*LIPA*	0.34 (T)	1.09 (1.07–1.12)
11q22.3	rs974819	*PDGF*	0.29 (T)	1.07 (1.04–1.09)
13q34	rs4773144	*COL4A1, COL4A2*	0.44 (G)	1.07 (1.05–1.09)
14q32.2	rs2895811	*HHIPL1*	0.43 (C)	1.07 (1.05–1.10)
15q25.1	rs3825807	*ADAMTS7*	0.57 (A)	1.08 (1.06–1.10)
17p13.3	rs216172	*SMG6, SRR*	0.37 (C)	1.07 (1.05–1.09)
17q11.2	rs12936587	*RASD1, SMCR3, PEMT*	0.56 (G)	1.07 (1.05–1.09)
17q21.32	rs46522	*UBE2Z, GIP, ATP5G1, SNF8*	0.53 (T)	1.06 (1.04–1.08)
21q22.13	rs9982601	*MRPS6*	0.15 (T)	1.19 (1.13–1.27)

Roberts R et al. J Am Coll Cardiol 60:1715–21, 2012

Ideally, a predictive test should precisely assign all screened individuals into disease-positive and disease-negative groups according to whether they will develop disease in the future or remain disease-free, respectively.

The ideal test: all people who develop disease are identified and no false positives exist

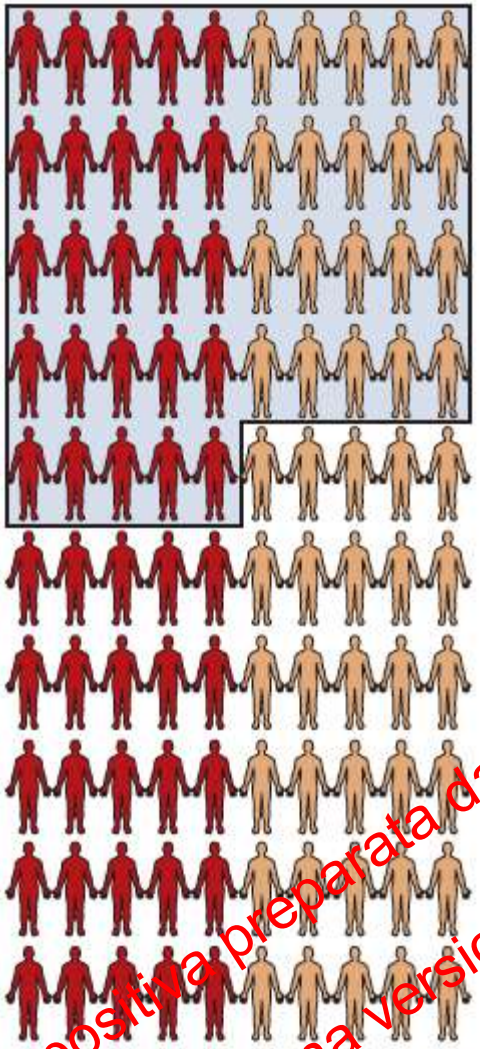


Sensitivity	100%
Specificity	100%
PPV	100%
NPV	100%

In a perfect scenario, all individuals that eventually develop disease test positive (sensitivity 100%), all individuals who remain disease-free test negative (specificity 100%), all individuals with a positive test later develop disease (positive predictive value 100%), and all individuals with a negative test remain disease-free (negative predictive value 100%).

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

A test in which half of those who will develop disease and almost half of those who will not develop disease are labeled 'test positive'

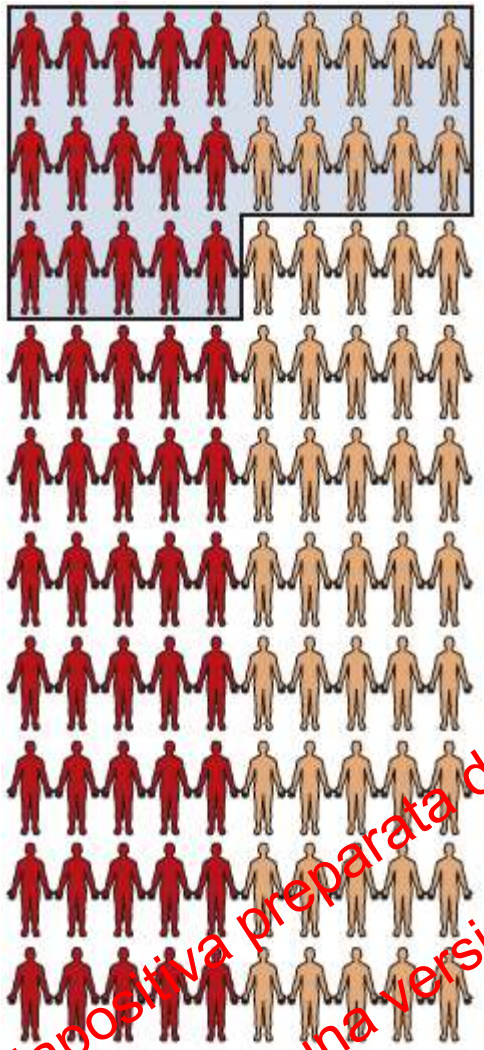


Sensitivity	50%
Specificity	60%
PPV	56%
NPV	55%

When a test relies on the presence or absence of a common variant of small effect would perform similarly to this hypothetical test and would provide limited discrimination for CHD, as carriage is neither sufficient nor necessary to develop CHD.

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

A test in which only a small fraction of those who develop disease are detected and a substantial number of those labeled 'test positive' remain disease-free.



Sensitivity	30%
Specificity	80%
PPV	60%
NPV	53%

When the test relies on the presence or absence of a single risk factor associated with a modest odds ratio for CHD risk such as the use of a systolic blood-pressure threshold of 160 mmHg would perform similarly to this hypothetical test.

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

Genetic Risk Score

For the prediction of complex diseases, genotypes at multiple SNPs are often combined into scores calculated according to the number of risk alleles carried.

Nine SNPs from 9 Loci of "cholesterol genes" and CVD

The Malmoe Diet and Cancer study (n=5,414; follow-up=10.6 yrs)

SNP	Gene	SNP Type	MAF	Percentage of Variance Explained	P Value†
LDL cholesterol					
rs693‡	*APOB*	Coding	0.48	0.9	1×10^{-11}
rs7575840	*APOB*	3′ Downstream	0.39	0.4	8×10^{-7}
rs4420638‡	*APOE* cluster	5′ Upstream	0.20	1.7	3×10^{-21}
rs12654264‡	*HMGCR*	Intronic	0.39	0.2	0.002
rs1529729‡	*LDLR*	Intronic	0.44	0.2	0.003
rs688	*LDLR*	Coding	0.42	0.1	0.04
rs11591147‡	*PCSK9*	Coding	0.01	0.5	7×10^{-7}
HDL cholesterol					
rs3890182‡	*ABCA1*	Intronic	0.13	0.2	0.003
rs1800775‡	*CETP*	5′ Upstream	0.49	2.5	2×10^{-29}
rs1800588‡	*LIPC*	5′ Upstream	0.21	0.8	4×10^{-10}
rs328‡	*LPL*	Coding	0.09	0.9	3×10^{-12}

Kathiresan S et al. N Engl J Med 358:1240-9,2008

Nine SNPs from 9 Loci of "cholesterol genes" and CVD
The Malmoe Diet and Cancer study (n=5,414; follow-up=10.6 yrs)

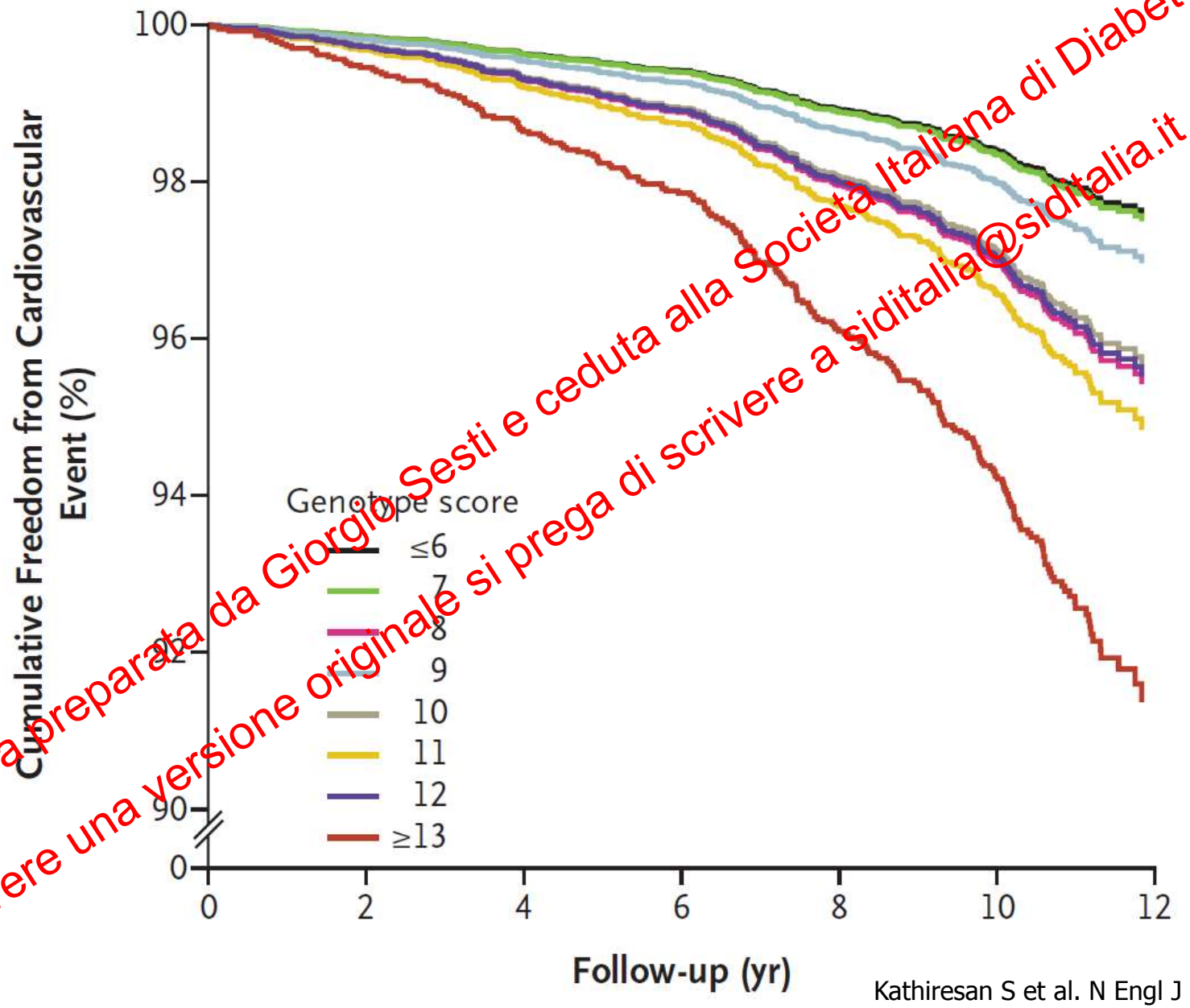
Table 3. Lipid Levels and Crude Incidence Rates of Cardiovascular Disease, According to Genotype Score*

Variable	Genotype Score†								P for Trend
	≤6 (N=122)	7 (N=309)	8 (N=574)	9 (N=894)	10 (N=913)	11 (N=726)	12 (N=465)	≥13 (N=229)	
LDL cholesterol (mg/dl)	152±41	152±37	156±38	159±38	163±37	165±38	168±41	171±36	2×10^{-18}
HDL cholesterol (mg/dl)	60±16	57±14	56±14	54±14	53±14	53±14	51±13	51±13	3×10^{-24}
Crude incidence rate per 1000 person-years	3.1	2.7	5.1	3.9	5.3	6.8	5.7	11.0	

Kathiresan S et al. N Engl J Med 358:1240-9,2008

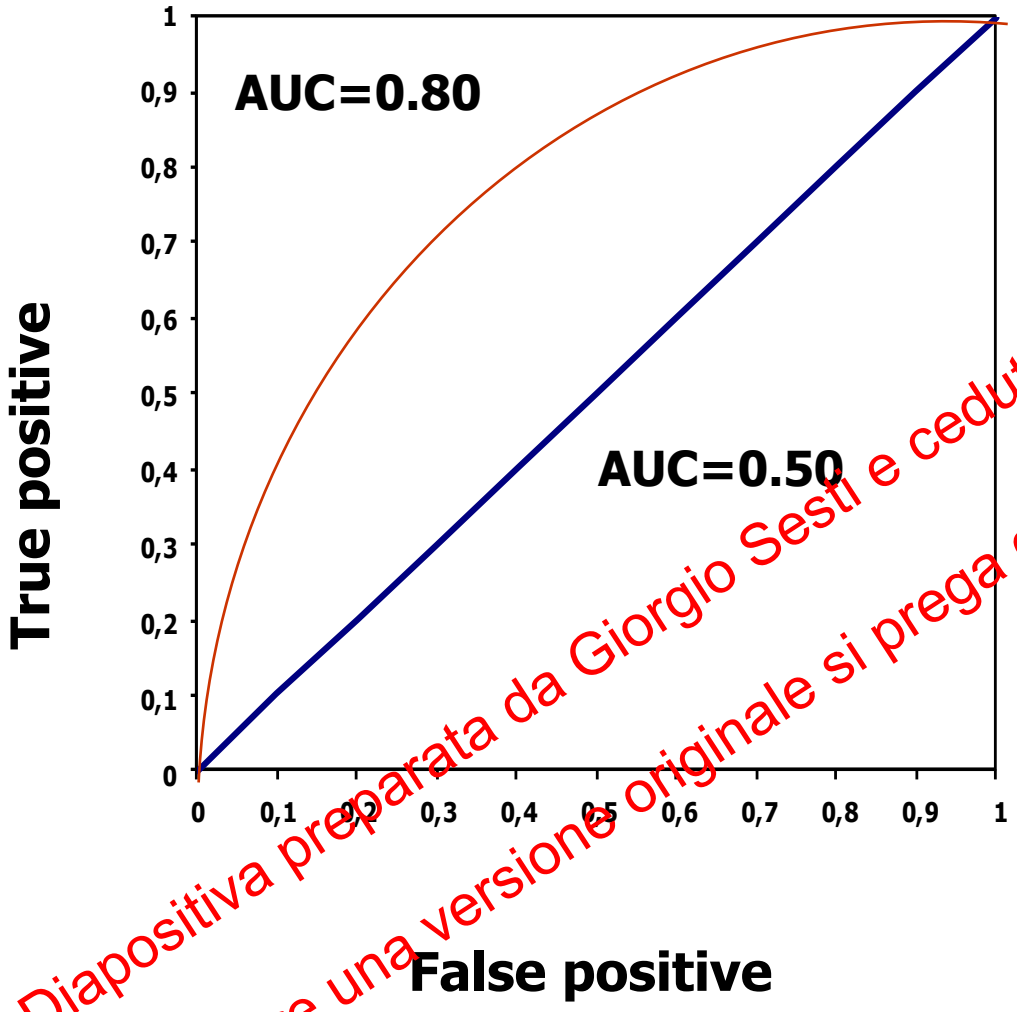
Nine SNPs from 9 Loci of "cholesterol genes" and CVD

Genotype score, per single unfavorable allele 1.15 (1.07–1.24); P<0.001



Kathiresan S et al. N Engl J Med 358:1240-9,2008

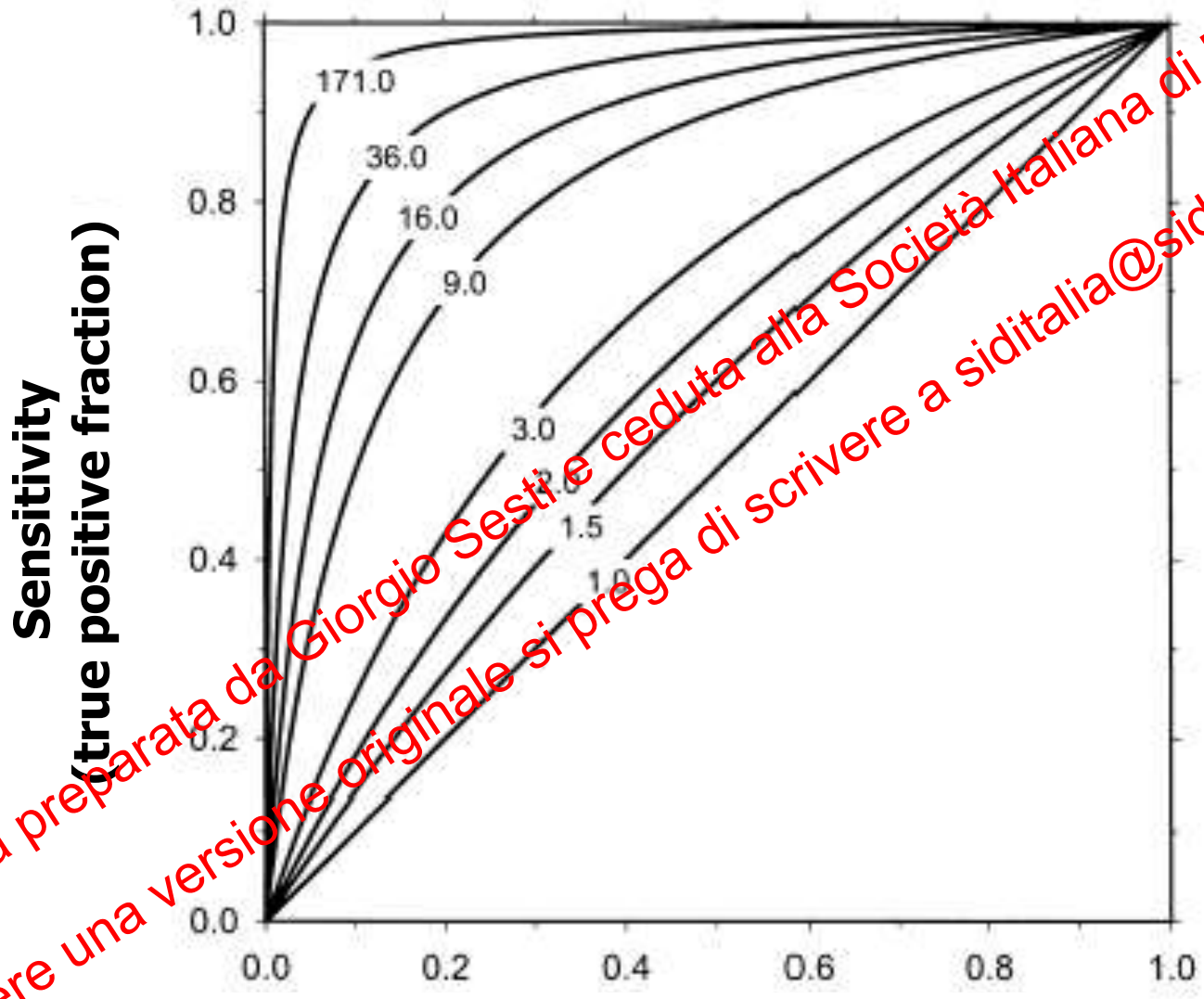
Receiver operating characteristic (ROC) curve



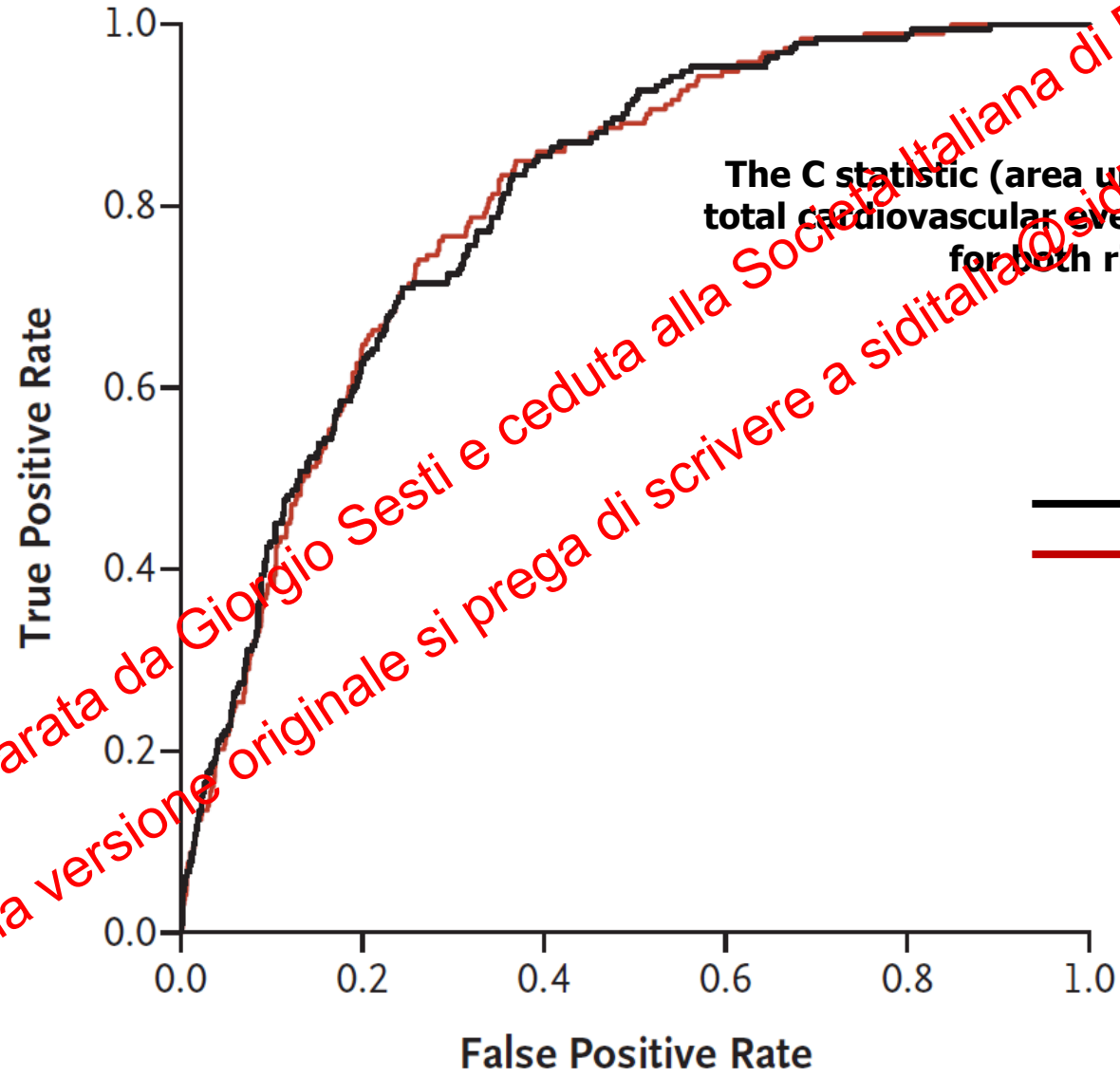
AUC = C-statistics

Probability that the predicted risk of person who will develop the event is greater than the predicted risk of a person who will not develop the event.

Area under the ROC Curve for Genetic Models Predicting CVD



Receiver-Operating-Characteristic (ROC) Curves for Incident Myocardial Infarction, Ischemic Stroke, or Death from Coronary Heart Disease during 10-Year Follow-up



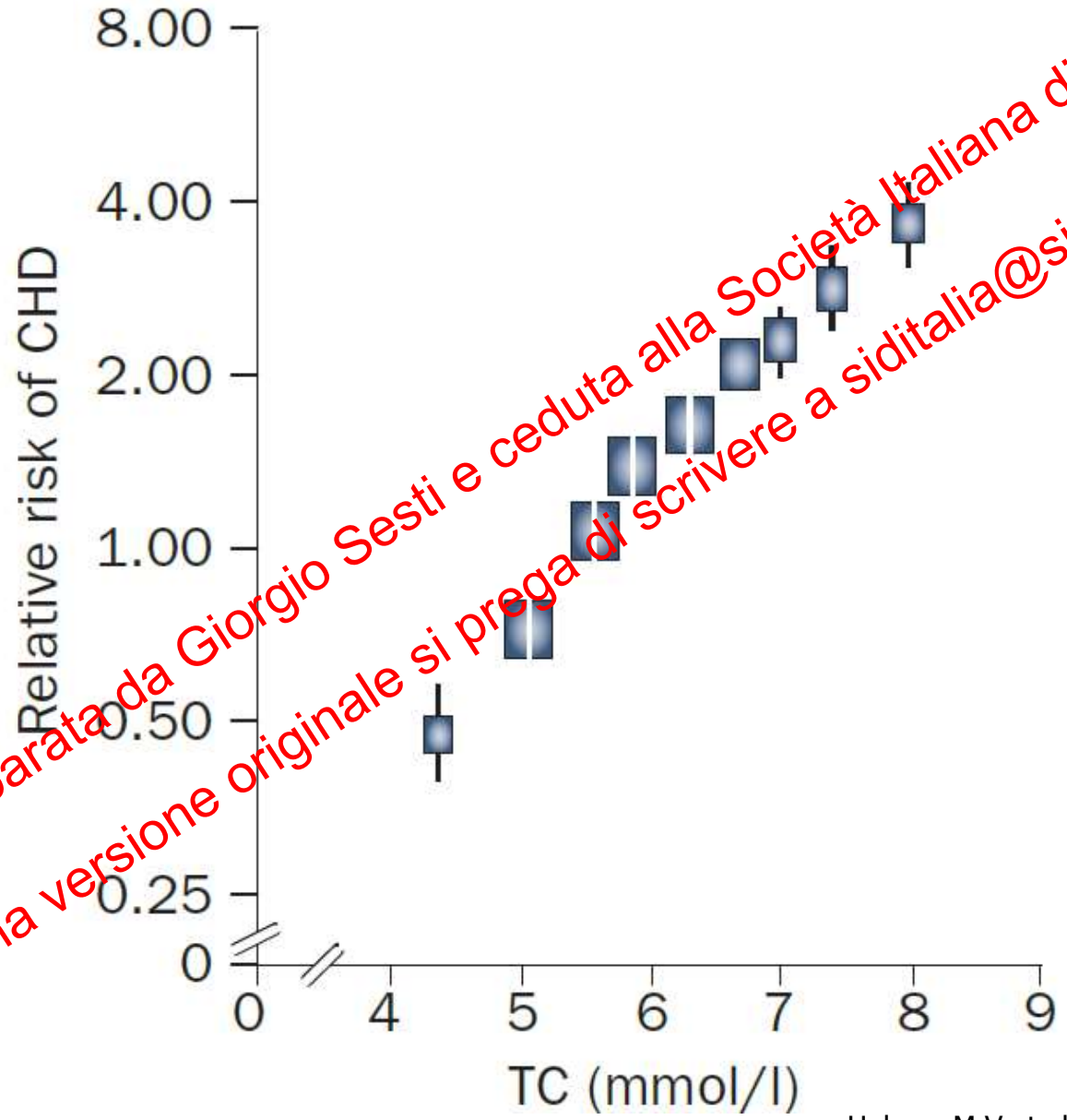
The C statistic (area under the ROC curve) for total cardiovascular events was the same (0.80) for both risk models.

Kathiresan S et al. N Engl J Med 358:1240-9,2008

Relevance of Rose's prevention paradox to genetic risk factors

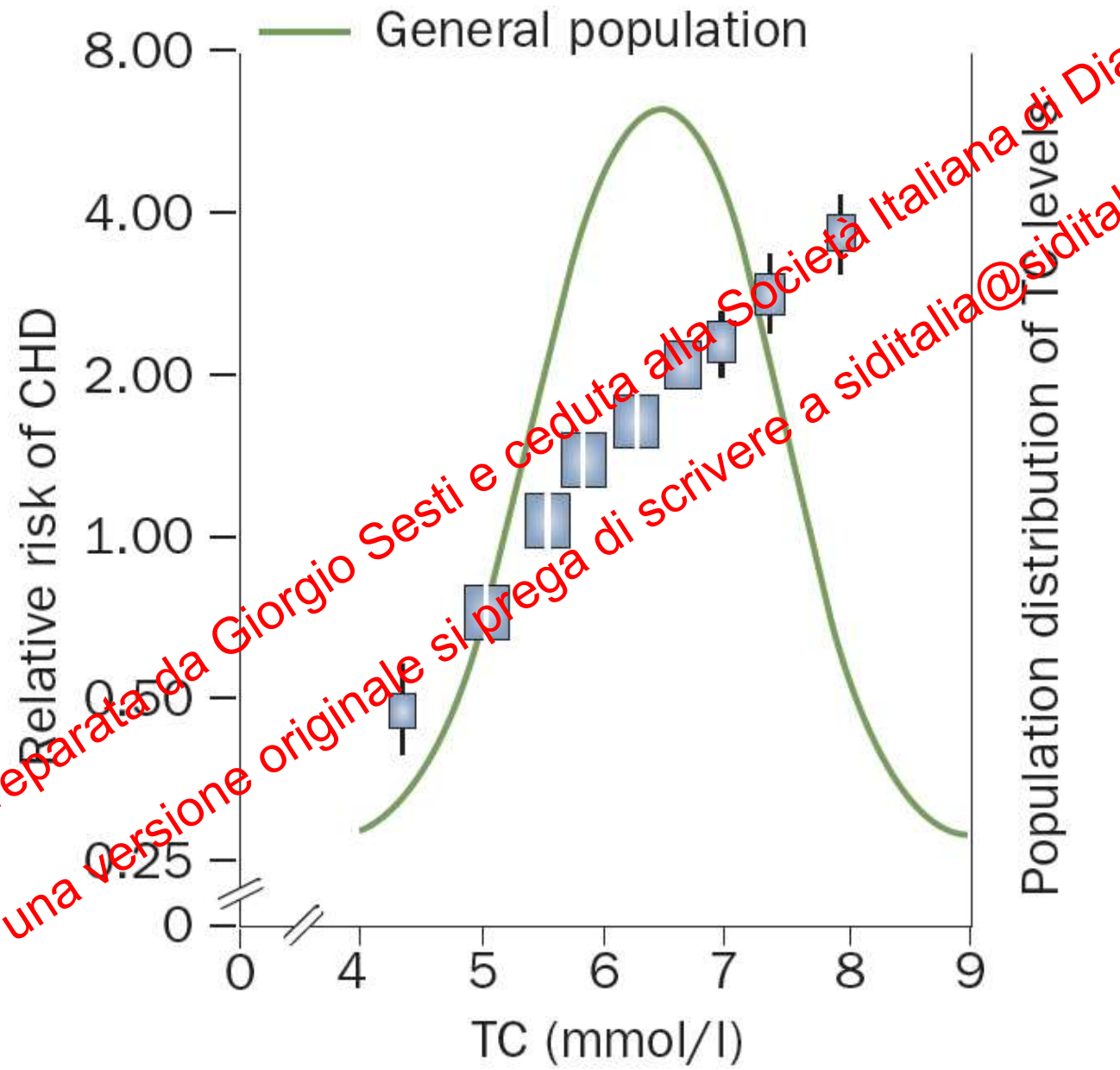
Geoffrey Rose determined that more cases of CVD occur among the majority at 'average risk' than among the minority at 'high risk', because of the linear association between risk factors and CHD events, and owing to the normal distribution of risk factors within populations.

Relationship between cholesterol level and CHD risk in the general population



Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

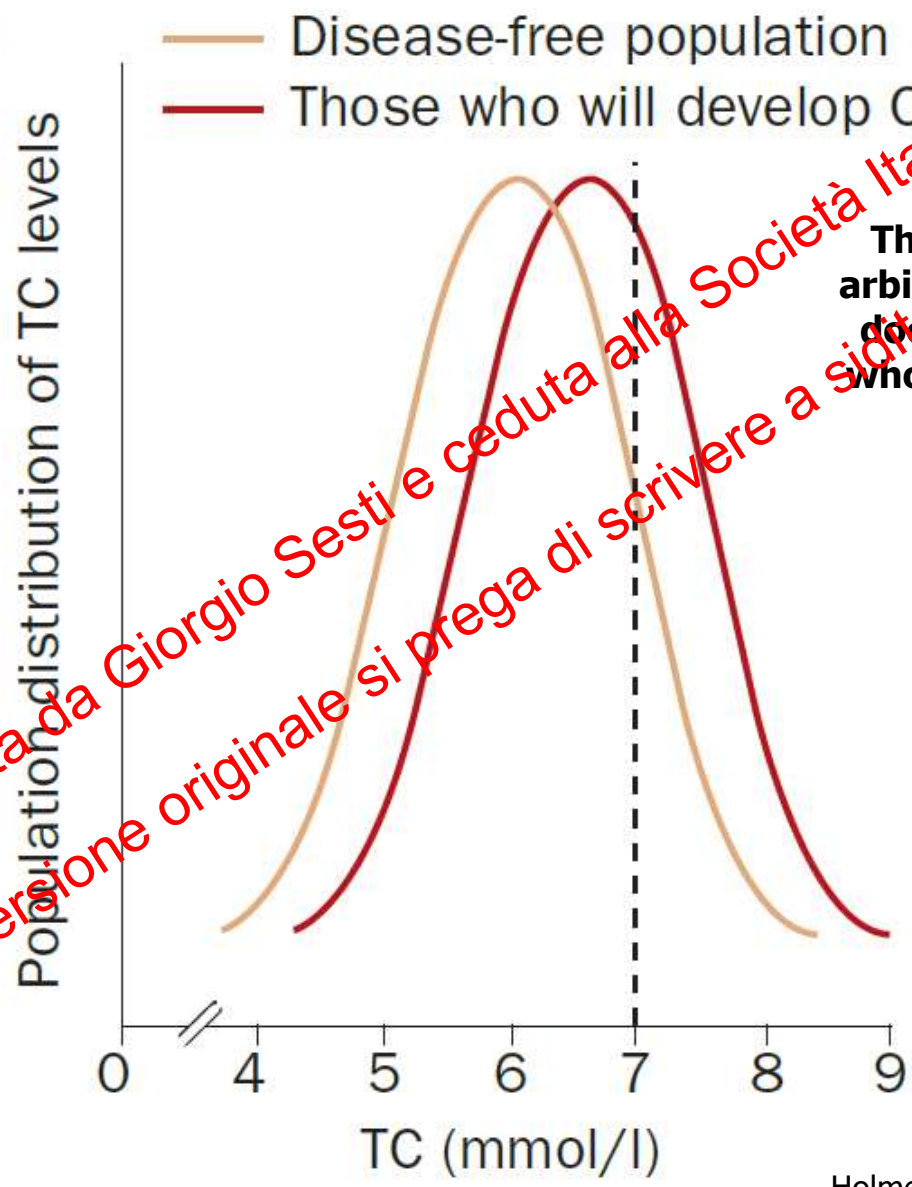
Approximate distribution of cholesterol in the general population, overlaid on the graph showing the relationship between cholesterol level and risk of CHD events



Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

The prevention paradox

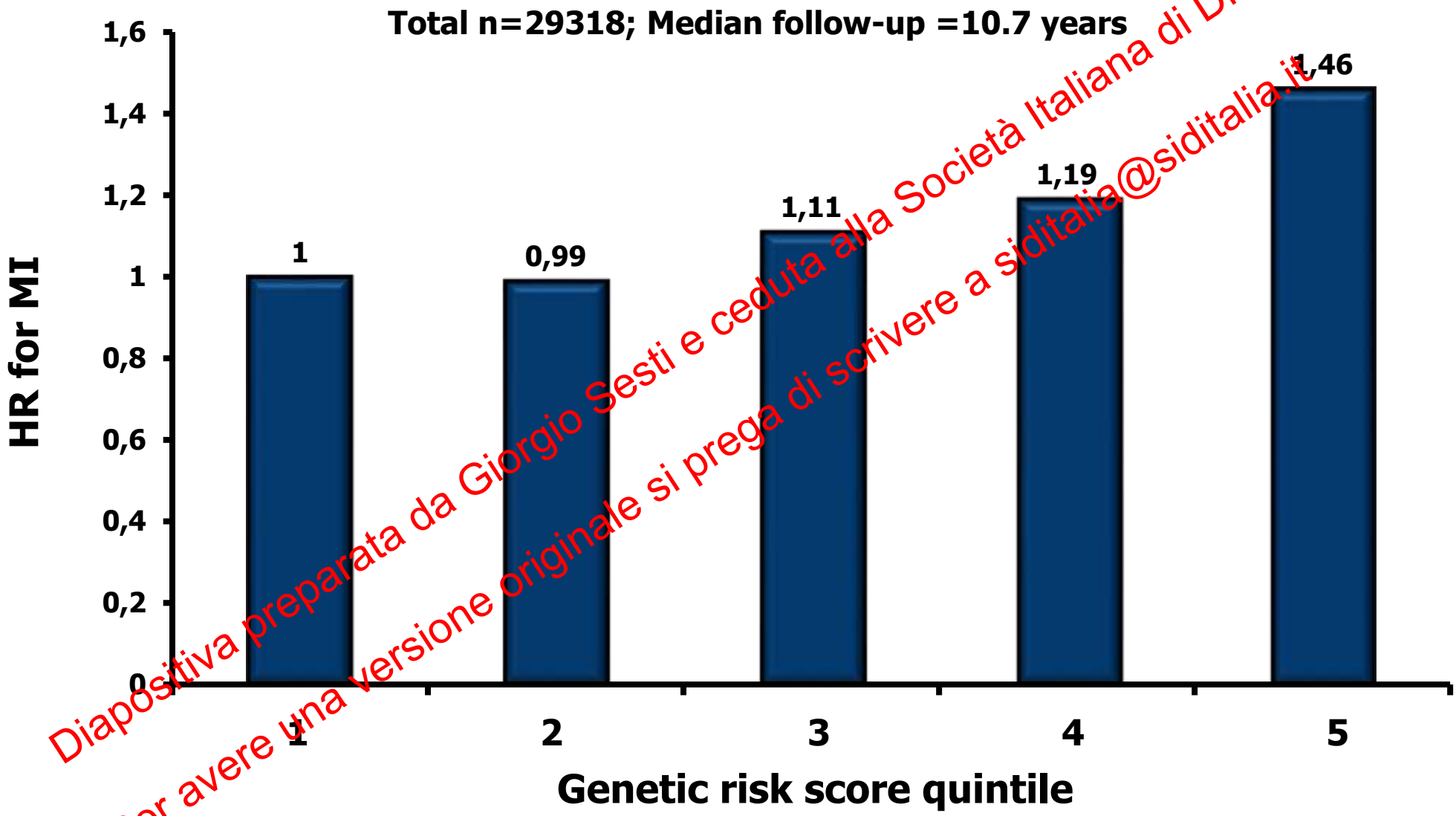
Depiction of the overlap in cholesterol levels between those who will later develop CHD and those remaining healthy in a typical prospective study



The vertical line illustrates that an arbitrary cut-point set at 7 mmol/l TC does little to distinguish individuals who develop disease from those who remain disease-free.

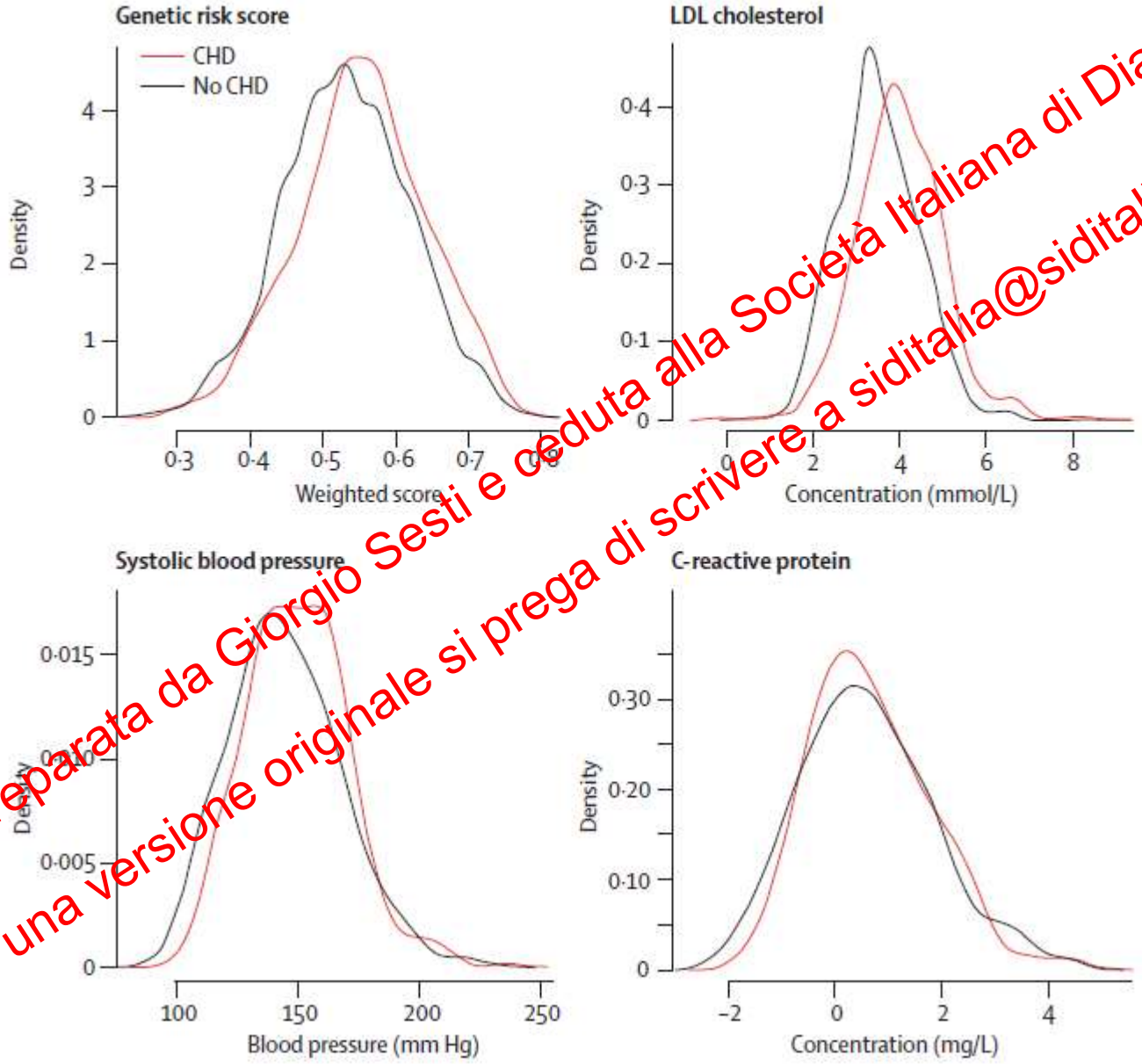
Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

Association between genetic risk score (13 SNPs) and incident myocardial infarction- FINRISK 1992, 1997, and 2002, the Health 2000 study, Malmo Diet and Cancer, and Malmo Preventive Project



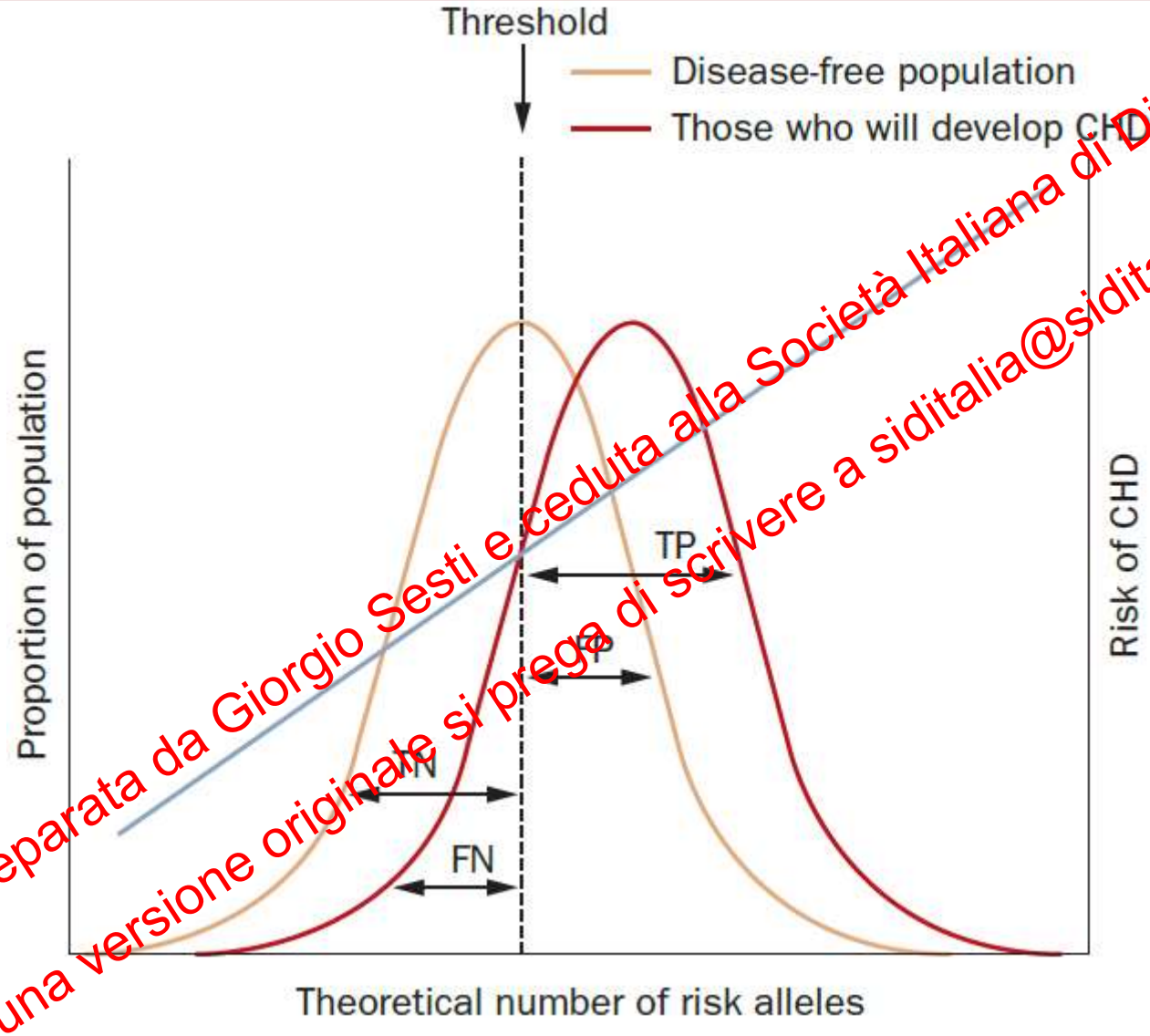
Ripatti S et al. Lancet 376: 1393–400, 2010

Distributions at baseline of genetic risk score, LDL, systolic BP, and CRP by 10-year incident coronary heart disease event status in FINRISK 1992 and 1997 cohorts



Ripatti S et al. Lancet 376: 1393–400, 2010

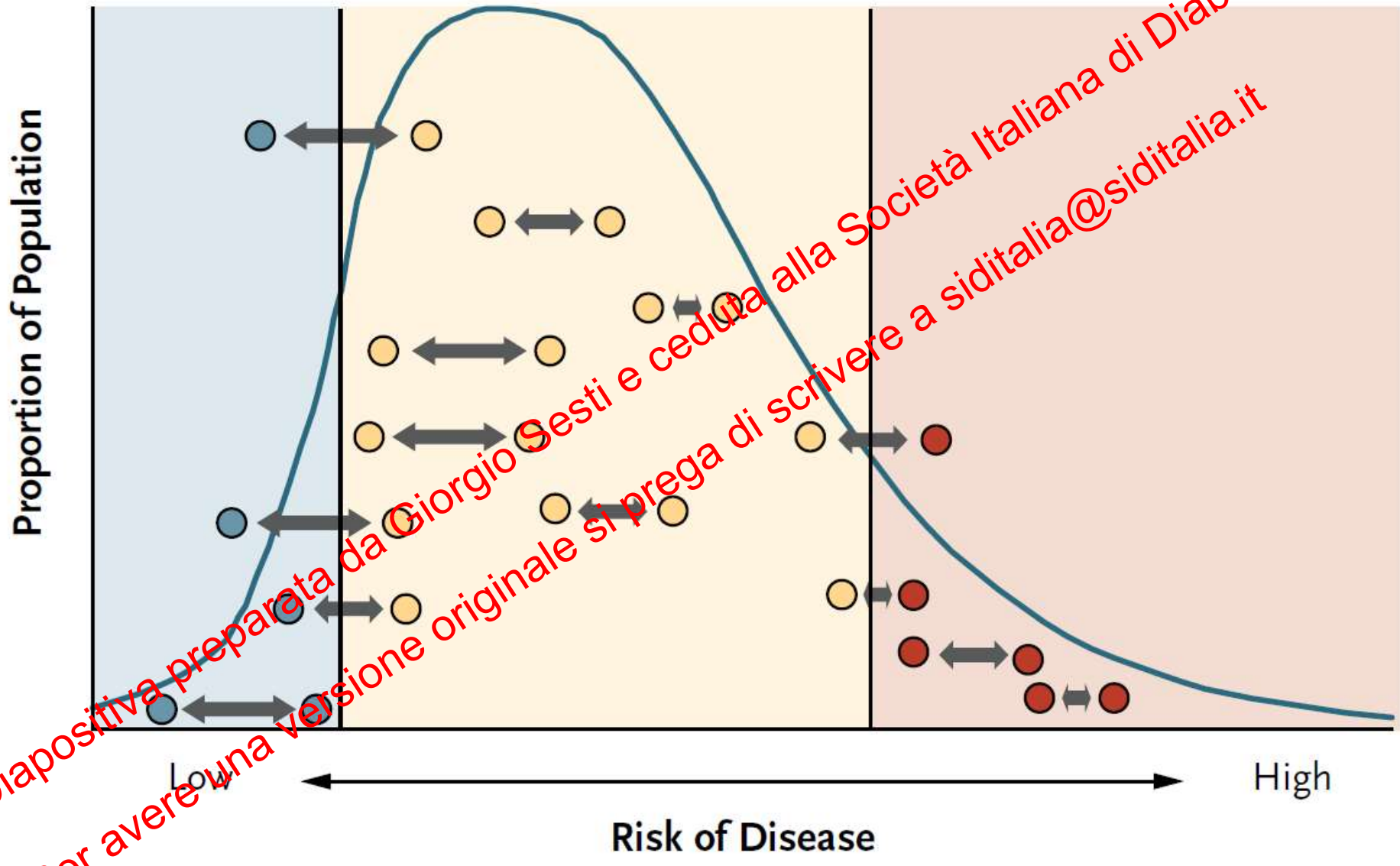
Relevance of Rose's prevention paradox to genetic risk factors



Since the frequency distribution of risk allele scores in a population is approximately normal, most cases of CHD in the population are expected among the majority of individuals with an intermediate number of genetic risk alleles.

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

Reclassification of Persons at Various Levels of Risk, According to Risk Thresholds



Manolio TA. N Engl J Med 363:166-76, 2010

Reclassification of individuals in the FINRISK 1992 and 1997 cohorts on the basis of 10-year predicted risk of coronary heart disease with and without genetic risk score

	Genetic risk score 0–5%	Genetic risk score 5–10%	Genetic risk score 10–20%	Genetic risk score >20%
Cases, by predicted risk*				
0–5%	82 (93%) →	6 (7%)	0	0
5–10%	10 (8%) ←	100 (84%) →	9 (8%)	0
10–20%	0	9 (5%) ←	136 (81%) →	22 (13%)
>20%	0	0	9 (7%) ←	129 (93%)
Non-cases, by predicted risk*				
0–5%	9224 (99%) →	121 (1%)	0	0
5–10%	179 (12%) ←	1149 (79%) →	131 (9%)	0
10–20%	0	130 (14%) ←	687 (80%) →	49 (6%)
>20%	0	0	54 (13%) ←	351 (87%)

	Individuals reclassified in table 5		Net reclassification improvement		
	Up →	Down ←	Value	95% CI	p value
Cases	37	28	0·018	−0·014 to 0·051	0·278
Non-cases	301	353	0·004	0·0001 to 0·008	0·038
Total	..	..	0·022	−0·010 to 0·055	0·182

Ripatti S et al. Lancet 376: 1393–400, 2010

Direct-to-consumer genetic tests are already available in many disease areas, but many have argued that this implementation is premature.

Direct-to-consumer genetic tests

- **In mid-2010, the US FDA advised commercial providers of genetic information that direct-to-consumer genetic tests are medical devices and might require FDA approval before commercial use;**

- **None of the major healthcare providers in Europe and the USA has adopted these tests for CHD risk prediction; however, individuals are able to purchase a 'genetic risk profile' through the commercial sector.**

Direct-to-consumer genetic tests

- **At present, issues relating to the performance and utility of genetic tests do not form a major part of medical training, and many clinicians might feel that they lack the necessary grounding required to effectively utilize such tests in a clinical setting;**

- **Concern about the use of DNA-based tests in relation to confidentiality, access to insurance products, and discrimination in the workplace.**

Population screening using common CHD-risk alleles

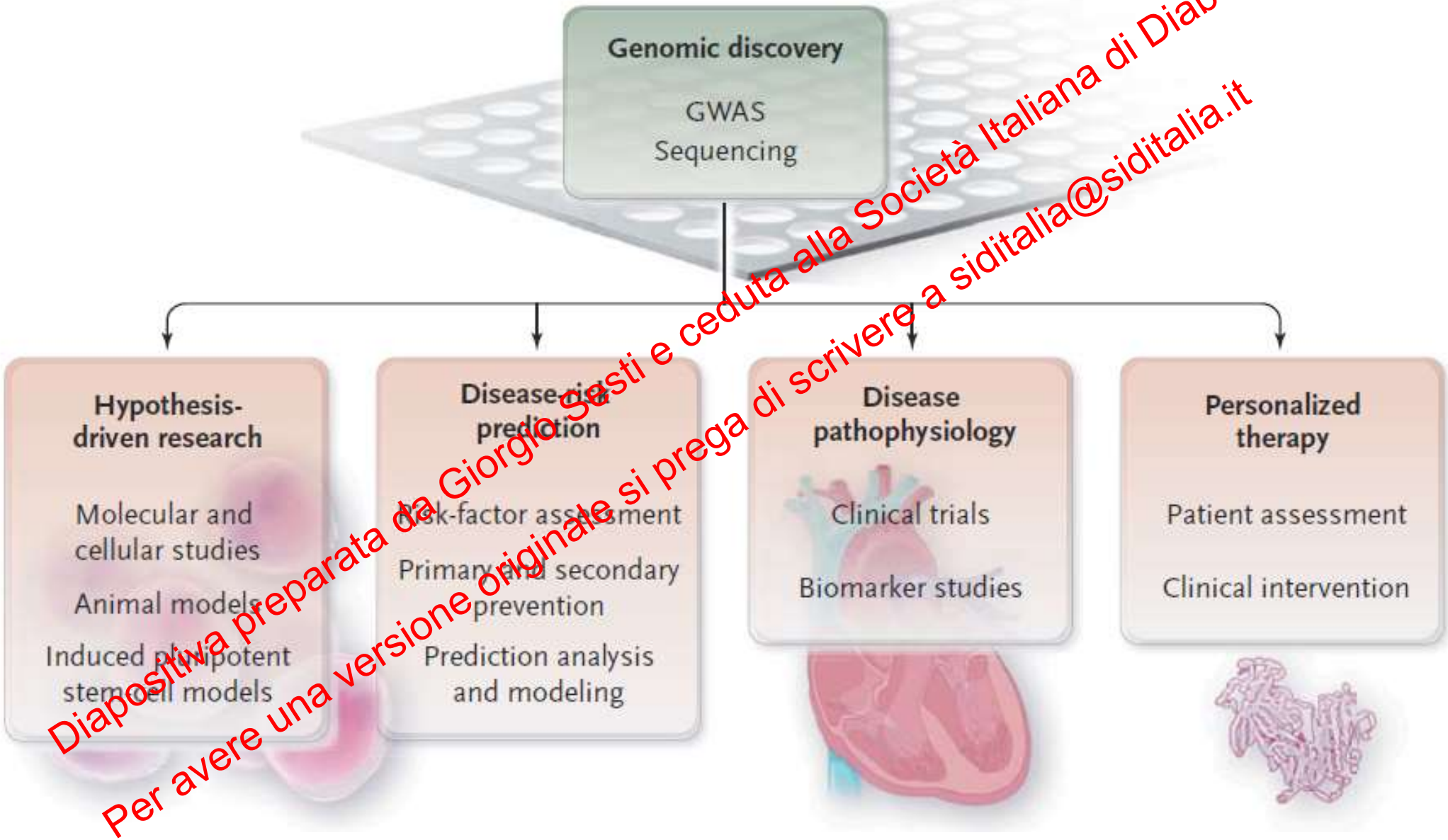
Metric	Multiple common variants of modest effect*			
	10-variant carrier	Individuals with ≥4 alleles	Individuals with <4 alleles	General population
Estimated CHD risk before and after statin intervention				
Lifetime risk before intervention§	76%	50%	34%	40%
Relative risk reduction with intervention‖	25%	25%	25%	25%
Risk after intervention	57%	38%	25.2%	30%
Absolute risk reduction with intervention	19%	12.5%	8.4%	10%
NNT	5	8	12	10
NNS on the basis of a population-wide approach				
Frequency of genetic exposure	0.00001	0.35	0.65	1
NNS (NNT/frequency of genetic exposure)	500,000	23	18	10

NNS, number needed to screen; NNT, number needed to treat.

Holmes M V et al. Nat Rev Cardiol 8, 207–221, 2011

With recent and near-future expiries of patents for the first statins, minimal improvement of risk stratification using genotype might not make genetic-based prediction a priority.

Investigative pathways leading from gene discovery to clinical application



O'Donnell C et al. N Engl J Med 365:2098-109, 2011

Ultimate Goal