Genetic variation in Estrogen Receptor alpha and risk of coronary artery disease: doubts and progress

Qualitative assessment of previous evidence and an updated meta-analysis confirms lack of association between the *ESR1* rs2234693 (*PvuII*) variant and coronary heart disease in men and women

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***Atherosclerosis*, 2009**

IMIM – FIIJT

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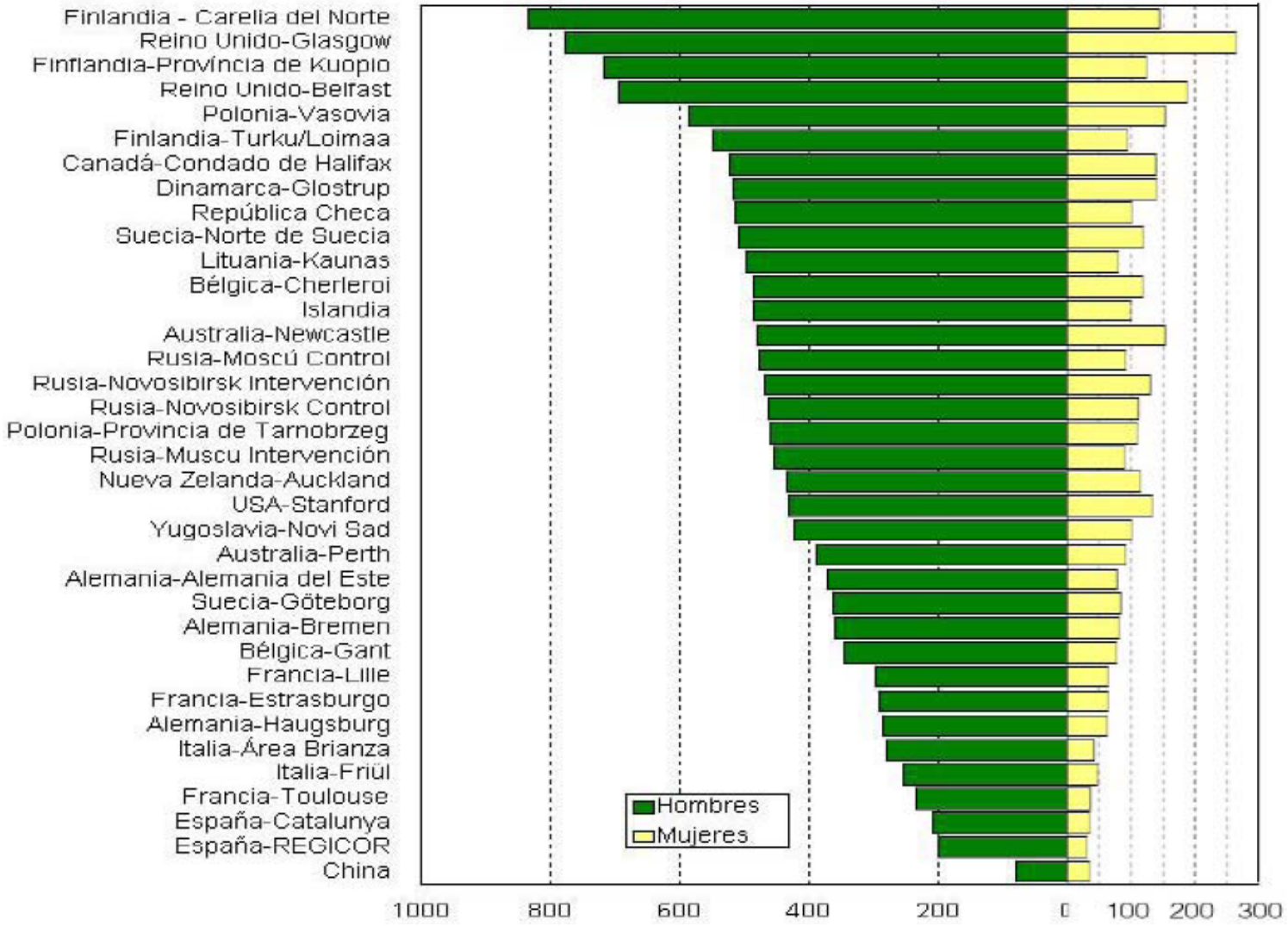
Gender as a risk factor for CAD



CVD Incidence (WHO – Monica)

Hypothesis:

- Elements of the sex hormone system might be responsible for gender differences in CAD risk
- 'Female' and 'male' sex hormones are expressed (in different quantities) by both sexes
- Inter-individual variation in sex hormone metabolism may give rise to inter-individual variation in CAD risk (regardless of gender)
- This may act through genetic variation in hormone-related genes
- **Genetic variation in the Estrogen Receptor alpha gene (*ESR1*) may modulate risk of CAD**

Broad range of genetic variation in *ESR1*

Estrogen Receptor alpha (ERa), encoded by *ESR1*:
>3,100 known single nucleotide polymorphisms known (dbSNP)

rs2234693 (*PvuII*) polymorphism, Intron 1

Focus on rs2234693 (*PvuII*)

Volume 15 Number 2 1987 Nucleic Acids Research

PvuII RFLP inside the human estrogen receptor gene

A.Castagnoli, I.Maestri, F.Bernardi and L.Del Senno

Centro di Studi Biochimici sul Morbo di Cooley, Università degli Studi di Ferrara, Italy

SOURCE/DESCRIPTION: 1.3 Kb insert of the human estrogen receptor cDNA in EcoRI site of the PBR322 (Green et al., 1986).

POLYMORPHISM: PvuII identifies five invariant bands at 13 ,5 , 3.3 , 2.8 ,1.0 Kb and a single two allele polymorphism with a band at either 1.5 and 0.7 Kb.

FREQUENCY: the 1.5 Kb band(see figure) is present in fourteen out of twenty unrelated Italian subjetcs with the frequency of 0.475.

NOT POLYMORPHIC for: BamHI, TaqI and MspI in at least 10 unrelated subjects.

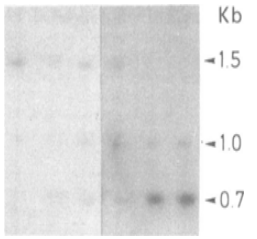
CHROMOSOMAL LOCALIZATION: 6 (Walter P. et al. 1985).

MENDELLIAN INHERITANCE: demonstrated in two Italian families.

PROBE AVAILABILITY: write to P.Chambon,Inst. Chim.Biol., 11,rue Humann, 67085 Strasbourg Cedex- France.

REFERENCE: Green S. et al. Nature (1986) 320, 134-139
Walter P. et al. Proc.Natl.Acad. Sci. USA (1985) 82, 7889-7893.

ACKNOWLEDGEMENTS: work supported by P.F.Ingegneria Genetica e Basi Molecolari Malattie Ereditarie CNR, cont. n° 86.00072.51.



[CANCER RESEARCH 49, 145–148, January 1, 1989]

Estrogen Receptor Expression in Human Breast Cancer Associated with an Estrogen Receptor Gene Restriction Fragment Length Polymorphism[1]

Steven M. Hill, Suzanne A. W. Fuqua, Gary C. Chamness, Geoffrey L. Greene, and William L. McGuire[2]

The University of Texas Health Science Center at San Antonio, Department of Medicine/Division of Oncology, San Antonio, Texas 78284-7884 [S. M. H., S. A. W. F., G. C. C., W. L. M.], and Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637 [G. L. G.]

ABSTRACT

Estrogen receptor (ER) content is a well-known predictor of clinical outcome in human breast cancer. The recent cloning of a human ER complementary DNA has made possible the characterization of the ER

an RFLP has also been identified in the human ER gene using the restriction enzyme *Pvu*II (11). The latter was described as a single, two-allele polymorphism consisting of fragments of approximately 1.5 and 0.7 kilobases.

breast cancer	myocardial infarction	bone mineral density
endometrial cancer	stroke	bone mass and geometry
Schizophrenia	cardiovascular risk factors	osteoporosis outcomes
Alzheimer's disease	arterial stiffness	body height
Cognitive functioning	high-density lipoprotein cholesterol	Polycystic ovary syndrome
vascular dementia	echocardiographic measurements	outcome of ovarian stimulation
methamphetamine induced psychosis	obesity and lipolysis	Endogenous estradiol
Migraine	metabolic syndrome	
	adiposity	
	fat mass	
	metabolic phenotypes	

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Intense but inconclusive research in Bone Mineral Density/Osteoporosis

Meta-analysis of previous association studies

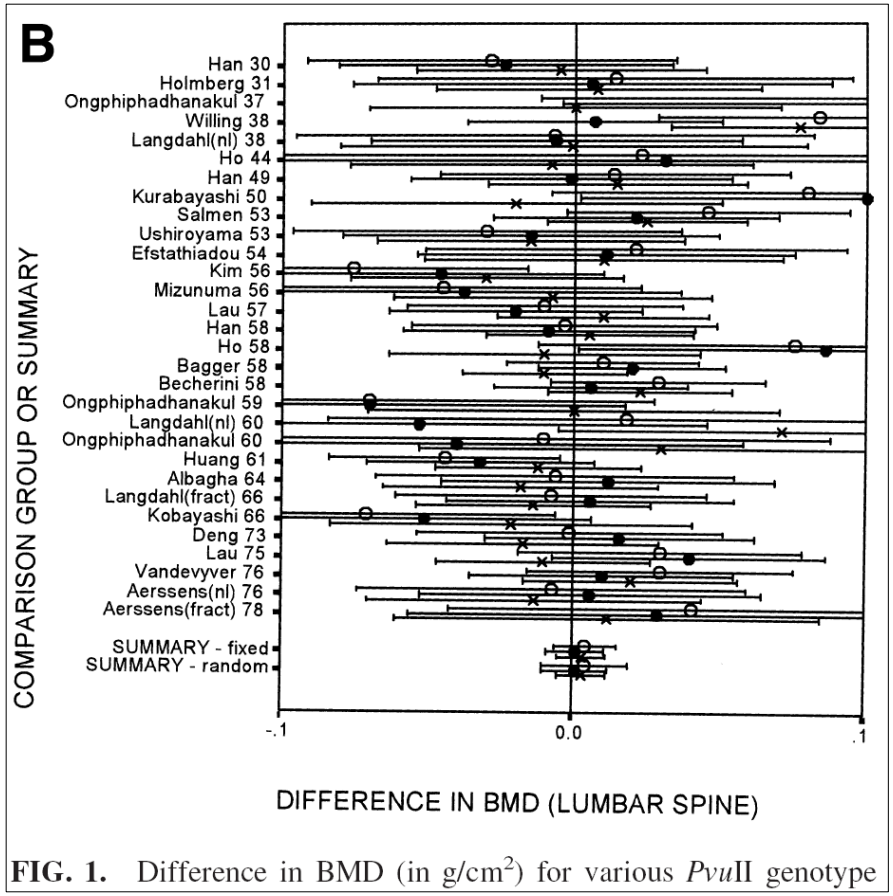


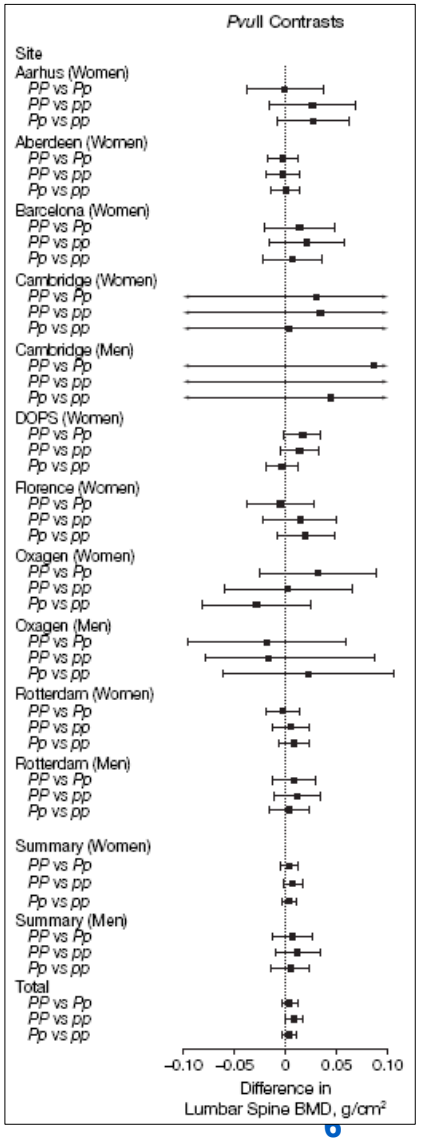
FIG. 1. Difference in BMD (in g/cm^2) for various *Pvu*II genotype

Ioannidis *et al. J Bone Miner Res* **17**, 2048-2060 (2002)

Meta-analysis of individual-level data involving standardized genotyping of 18,917 individuals in 8 European centers.

N~19,000

(Study restricted to 1 variant)



Ioannidis *et al. JAMA* **292**, 2105-2114 (2004)

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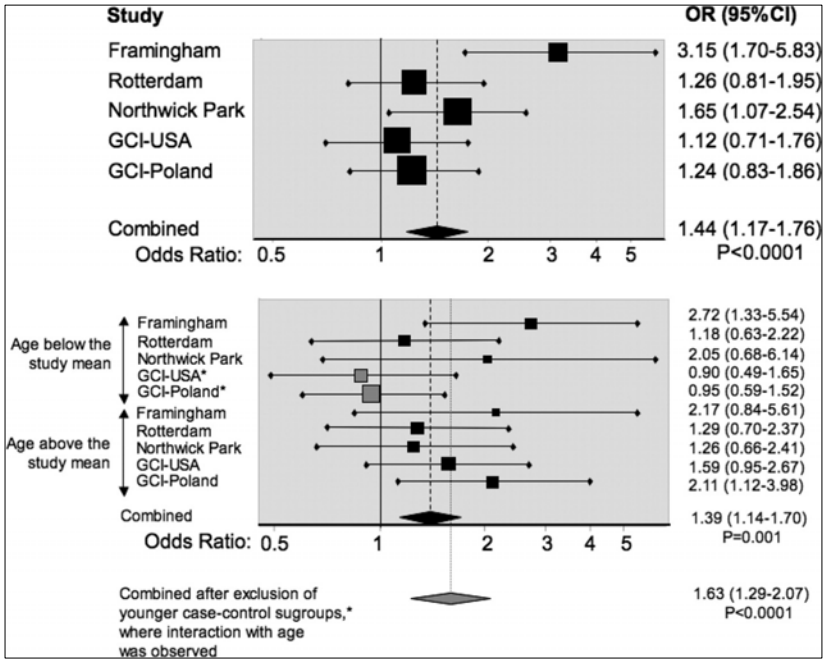
More inconclusive results for Coronary Artery Disease

Genotype CC of ESR1 c.454-397T>C and nonfatal myocardial infarction in men from 5 studies

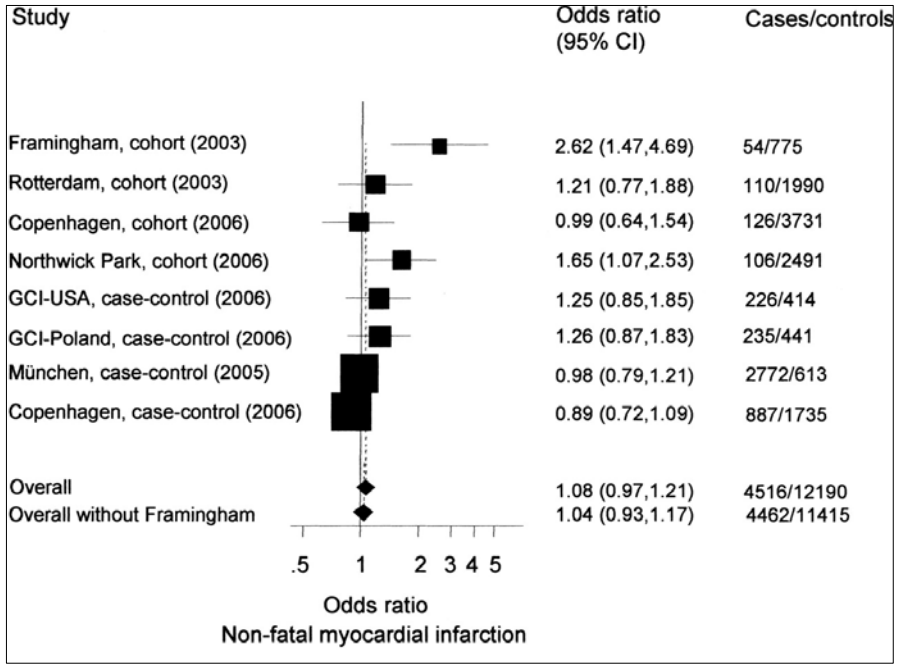


Shearman, A. M. et al. Circ Res 2006;98:590-592

N_T~7,000

Positive association between *PvuII* and CAD

Meta-analysis in men of ESR1 IVS1-397T/C CC vs CT/TT genotype on risk of fatal and nonfatal MI from 6 previous and the 2 present studies using fixed-effects model



Kjaergaard, A. D. et al. Circulation 2007;115:861-871

N_T~16,000

No association between *PvuII* and CAD

Our study

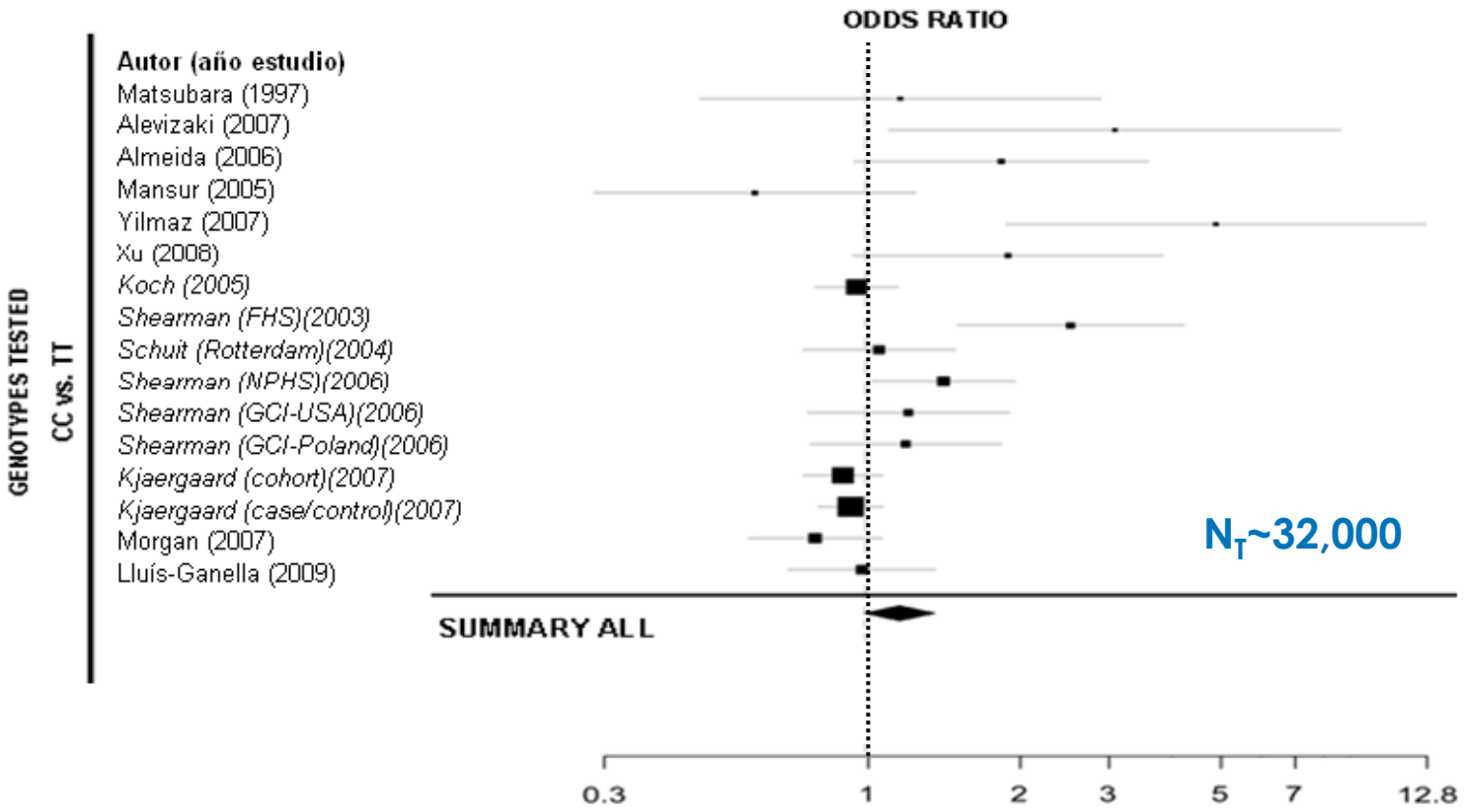
AIMS:

1. **Test for association between this variant and risk of CAD in a population from the region of Girona (The REGICOR Study; n~420 cases of MI and 1270 controls)**

2. **Summarise all evidence to date on this question (meta-analysis, n~32,000)**

3. **Investigate why the results of previous studies have been inconsistent (qualitative assessment)**

Large meta-analysis of previous evidence



Genotype	OR (95%CI)	Association p-value	Heterogeneity
TT	1	-	-
TC	1.06 (0.96-1.18)	0.243	0.013
CC	1,17 (1,00-1,32)	0,055	0,00003

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Which factors could explain this inconsistency between studies?

- **Meta-regression: used to "adjust" the meta-analysis for various study characteristics to see what causes between-study heterogeneity**

- **Heterogeneity could not be explained by differences between studies in terms of:**
  - clinical outcomes measured (MI or CAD)
  - study design (case-control or cohort design)
  - gender
  - sample size

- **But some studies 'feel' more convincing than others**

Guidelines for performing and reporting genetic association studies

- **NCI-NHGRI Working Group on Replication in Association Studies**

NATURE|Vol 447|7 June 2007 nature

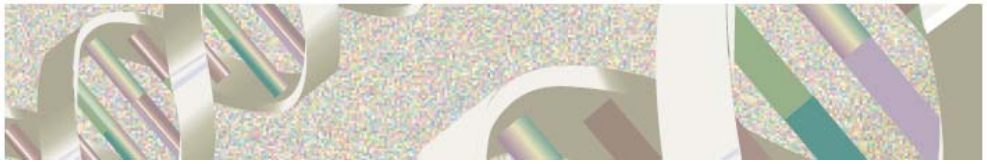
FEATURE

Replicating genotype–phenotype associations

What constitutes replication of a genotype–phenotype association, and how best can it be achieved?

NCI-NHGRI Working Group on Replication in Association Studies

The study of human genetics has recently undergone a dramatic transition with the completion of both the sequencing of the human

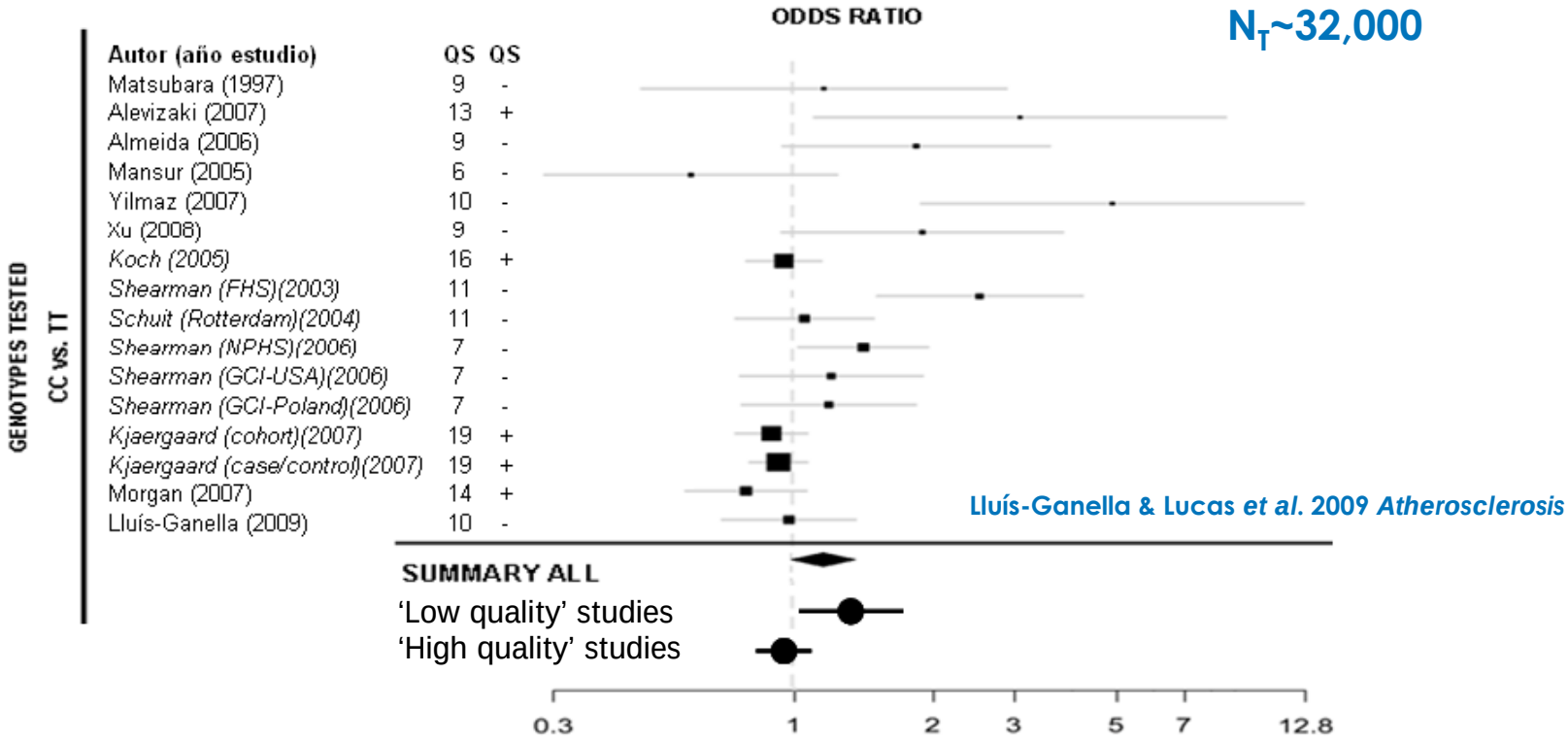


- **Association studies should provide datails on:**
  - study characteristics (e.g. patient sources; clinical characteristics)
  - genotyping quality control (e.g. well described; internal controls used)
  - methods and results (described well enough to replicate experiment)
  - replication and validation (e.g. in independent samples)

Guidelines for performing and reporting genetic association studies

Author (Publication year) Reference / Question/Condition	Matsubara 1997 [4]	Alevizaki 2007 [5]	Almeida 2006 [6]	Mansur 2005 [7]	Yilmaz 2007 [8]	Xu 2008 [9]	Koch 2005 [10]	Shearman 2003 [11]	Schuit 2004 [12]	Shearman 2006 [13]	Kjaergaard 2007 [3]	Morgan 2007 [14]	*Current study 2009*
Study information													
1 A detailed description of the study design and its implementation	•	•	•	•	•	•	•	•	•		•	•	•
2 The source of cases and controls or cohort members, if based on cohort design	•	•	•		•	•	•	•	•	•	•	•	•
3 Methods for ascertaining and validating affected or unaffected status and reproducibility of classification	•	•	•		•	•	•	•	•	•	•	•	•
4 Participation rates for cases, controls or cohort members		•						•	•		•	•	
5 Presentation of case and control selection in a flow chart		•						•			•		
6 Initial table comparing relevant characteristics of cases and controls	•		•	•	•	•	•		•		•	•	•
7 Success rate for DNA acquisition											•		
Data issues													
8 Statement on availability of results and data													
9 Links to supplemental online resources and database accession numbers										•	•		
Genotyping and quality control procedures													
10 Sample tracking methods, such as barcoding, to ensure accuracy of analysis													
11 Description of genotyping assays and protocols	•	•	•	•	•	•	•	•	•		•		•
12 Description of genotyping calling algorithm													
13 Genotype quality control design for samples		•					•			•	•	•	
14 External control samples from standard accepted sets (such as HapMap)													

Study quality explains heterogeneity in results



Lluís-Ganella & Lucas *et al.* 2009 *Atherosclerosis*

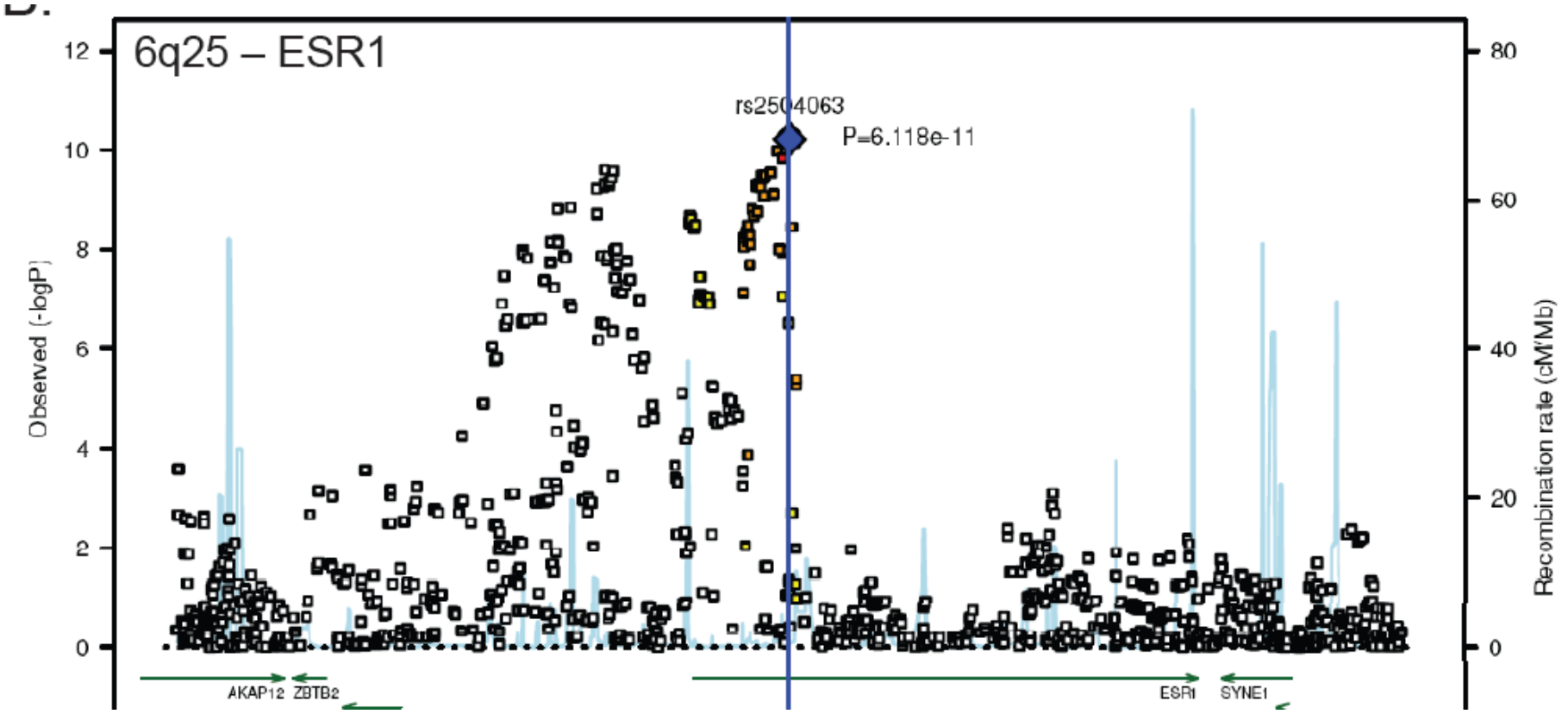
	Low Quality Studies			High Quality Studies		
Genotype	OR (95%CI)	P-value	Heterogeneity	OR (95%CI)	P-value	Heterogeneity
TT	**1**	**-**	**-**	**1**	**-**	**-**
CC	**1,37 (1,08-1,74)**	**0,01**	**0,0055**	**0,93 (0,82-1,05)**	**0,25**	**0,1565**

Conclusion: No association between this SNP and CAD. But what about the rest of *ESR1*?

GWAS results for Bone Mineral Density in *ESR1*

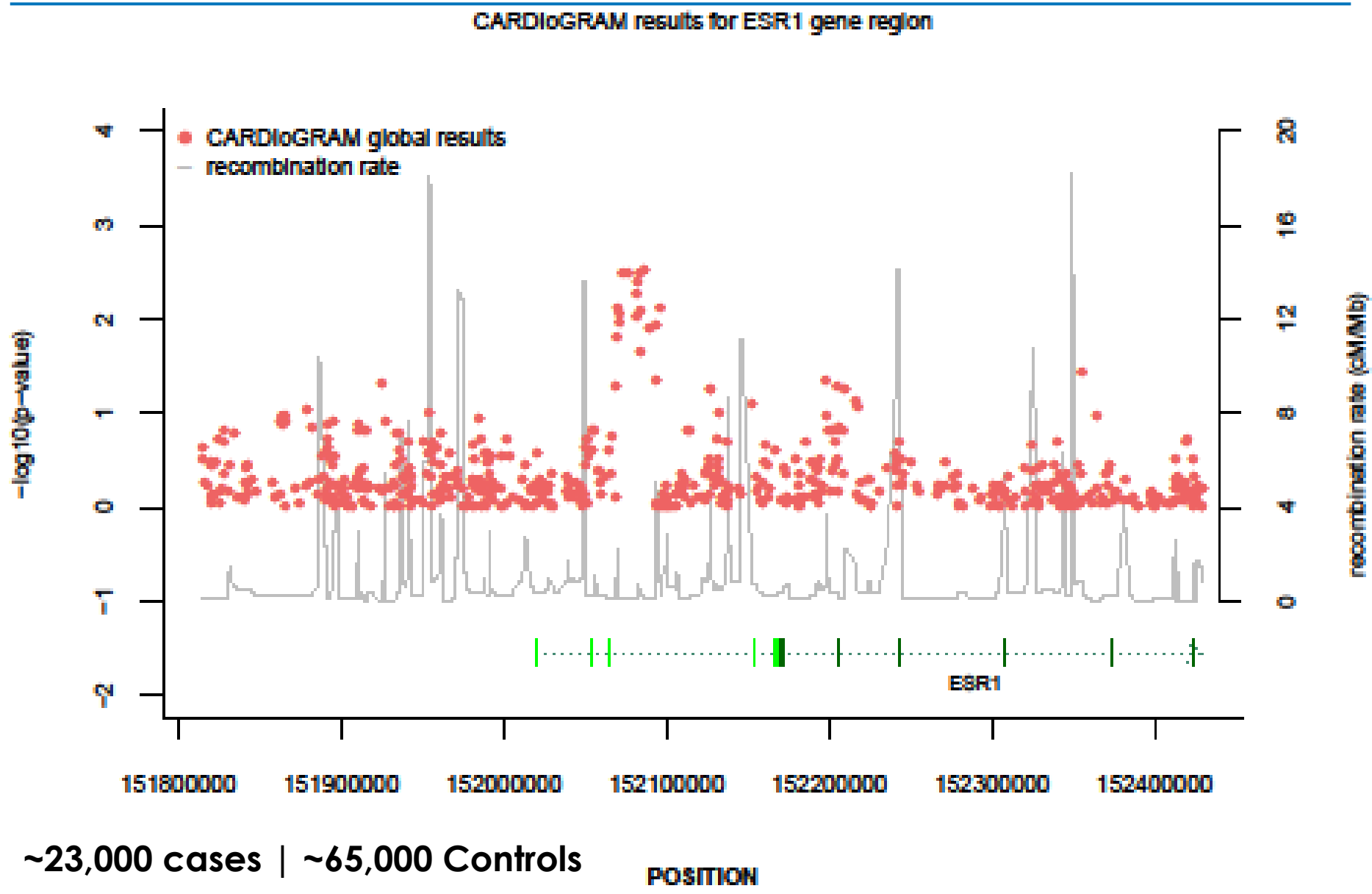
"Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies" (Rivadeneira et al., Nature Genetics, Oct 2009)

- **strongest results lie in a region that which does not contain *PvuII***



CAD?

GWAS results for *ESR1* in MI/CAD – The CARDIoGRAM Consortium



~23,000 cases | ~65,000 Controls

Thanks to ...

The REGICOR Investigators

- Joan Sala
- Jaume Marrugat
- Roberto Elosua
- Rafel Ramos
- Carla Lluís
- Isaac Subirana

REGICOR
Registre Gironí del Cor

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