

S. Matthijs Boekholdt · Ron J. G. Peters
Katerina Fountoulaki · John J. P. Kastelein
Eric J. G. Sijbrands

Molecular variation at the apolipoprotein B gene locus in relation to lipids and cardiovascular disease: a systematic meta-analysis

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Abstract Apolipoprotein B (apoB) is the sole protein component of low-density lipoprotein (LDL) and is thought to play an important role in atherogenesis. We performed a meta-analysis of the associations between the three most frequently investigated polymorphisms (*Xba*I, signal peptide insertion/deletion, *Eco*RI) in the apolipoprotein B (APOB) gene, lipid parameters, and the risk of ischemic heart disease (IHD). We restricted our analysis to Caucasians. Homozygotes for the *Xba*I X+ allele had significantly elevated levels of LDL cholesterol (LDL-C) and apoB, but a decreased risk (OR=0.80; 95%CI: 0.66–0.96) of IHD. Homozygosity for the signal peptide deletion allele was associated with similarly increased levels of LDL-C and apoB, and with an increased risk of IHD (OR=1.30; 95%CI: 1.08–1.58). Subjects homozygous for the rare *Eco*RI allele had significantly decreased levels of total and LDL cholesterol, but unaltered risk of IHD. We conclude that all three polymorphic apoB sites are associated with altered lipid levels, but not necessarily with a consistently altered risk of IHD. These data suggest that the relationship between apoB levels, hypercholesterolemia and IHD risk cannot have a simple molecular basis in the apoB gene.

Introduction

Elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) are associated with an increased risk of coronary artery disease (Castelli et al. 1986). Apolipoprotein B (apoB) is the sole protein component of this particle, and plasma levels of apoB are also associated with cardiovascular disease. Indeed, evidence is accumulating that apoB may be a better risk indicator for cardiovascular events than LDL-C (Sniderman et al. 2003). In a large survey of 175,553 subjects, the apoB concentration was associated with the risk of fatal myocardial infarction, even after adjustment for total cholesterol (Walldius et al. 2001). LDL-C levels are less predictive of cardiovascular events in patients treated with statin therapy. Hence, apoB-guidance of statin treatment may more accurately predict the effect on cardiovascular risk (Miremadi et al. 2002; Sniderman et al. 2003).

Several mechanisms may explain the role of apoB in atherogenesis. ApoB serves as the ligand for LDL-receptor mediated clearance of LDL. Mutations in the APOB gene cause a rare dominant disorder, familial defective apolipoprotein B-100, which is characterized by elevated LDL-C levels and an increased risk of ischemic heart disease (IHD). These rare genetic variants cannot explain the variation of apoB in the general population. Less penetrant but more prevalent variants could have a much larger population-attributable effect. As a consequence, a plethora of studies have assessed a wide variety of APOB polymorphisms with inconsistent results, possibly because of the lack of adequate statistical power, selection bias, population diversity, or genetic admixture (Ioannidis et al. 2001). A meta-analysis may avoid some of these methodological difficulties. Therefore, we have performed a systematic meta-analysis of population-based studies investigating APOB polymorphisms and their association with either lipid parameters or the risk of IHD.

S. M. Boekholdt (✉) · R. J. G. Peters
Department of Cardiology, Room F3-241,
Academic Medical Center,
PO Box 22660, 1100 DD Amsterdam, The Netherlands
Tel.: +31-20-5666834, Fax: +31-20-5669747,
e-mail: s.m.boekholdt@amc.uva.nl

K. Fountoulaki
Department of Internal Medicine, University Hospital of Larissa,
Larissa, Greece

J. J. P. Kastelein
Department of Vascular Medicine, Academic Medical Center,
Amsterdam, The Netherlands

E. J. G. Sijbrands
Department of Internal Medicine, Erasmus Medical Center,
Rotterdam, The Netherlands

Table 1 Nomenclature for the investigated APOB polymorphisms. Allele frequencies are based on the pooled control groups of all studies investigating genotype-IHD relationships. The genotype distributions in the pooled control groups are given in absolute numbers

Polymorphism	Allele	Exon	Location	Nucleotide	Amino acid	Allele frequency	Rare allele		Common allele Homozygotes
							Homozygotes	Heterozygotes	
<i>Xba</i> I	X-	26	Codon 2488	ACC	Thr	0.506	X+X+	X+X-	X-X-
	X+			ACT	Thr	0.494			
Signal peptide	insertion	1		CTG GCG CTG	Leu-Ala-Leu	0.680	DelDel	InsDel	InsIns
	deletion			-	-	0.320			
<i>Eco</i> RI	E+	29	Codon 4154	GAA	Glu	0.802	E-E-	E+E-	E+E+
	E-			AAA	Lys	0.198			

Materials and methods

Nomenclature

Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Literature search

We identified all population-based studies of APOB polymorphisms and their association with lipid parameters and the risk of IHD. The literature was scanned by a formal search of the MEDLINE electronic database. Search terms that were used were both MESH terms and (part of) the text words "acute coronary syndromes", or "myocardial infarction", or "coronary artery disease", or "ischemic heart disease", in combination with "apolipoprotein B", in combination with "polymorphism", or "mutation", or "genetics". The search results were subsequently limited to "human" and "English language". Reference lists of retrieved articles were scanned for additional potentially relevant publications. In addition, for each retrieved publication an electronic "cited reference search" was performed (Web of Science version 4.1.1, Institute for Scientific Information 2000, <http://www.isinet.com/isi/products/citation/wos/>), identifying all papers citing the index publication.

We checked whether all generated data described in the Methods section were reported in the Results section of each paper. If obtained data were not shown in the article, the principal investigator was contacted and asked to provide the data. Authors were also contacted in case of a potential patient overlap between publications.

Selection criteria

We aimed to reduce publication bias by contacting investigators to request unpublished data. Because it was virtually impossible to obtain unpublished data from studies published more than 10 years ago, we restricted our analysis to articles published after 1992. Extension of this period to before 1992 would result in the inclusion of many studies with incomplete data (data not available digitally, only in old digital format, investigator moved, retired, etc.) and would thus introduce publication bias. Only full-length articles in peer review journals were included if published between 1992 and January 2002. We limited our analysis to the three most extensively studied APOB polymorphisms, i.e., the *Xba*I, signal peptide insertion/deletion, and *Eco*RI polymorphisms. Moreover, we restricted our analysis to studies performed in Caucasian populations, because genotype distributions may differ substantially between different ethnicities (Peters and Boekholdt 2002). If ethnicity was not explicitly reported, studies were also included if they were performed on European and Australian populations. Data on

subjects from other countries were excluded, as were data from articles in which ethnicity and the country were not reported.

Studies were included if (1) they reported the prevalence of one of these polymorphisms in at least one group of patients with IHD and in an appropriate group of population-based controls without cardiovascular disease and representative for the population from which the cases were recruited, or (2) they reported data on both an APOB polymorphism and a lipid parameter in a sample of unrelated individuals. We limited our analysis to the following four plasma lipid parameters: total cholesterol (TC), triglycerides, LDL-C, and apoB. IHD was defined as (1) myocardial infarction, (2) unstable angina, (3) stable angina, or (4) angiographic evidence of coronary artery disease. Data on subjects with lipid disorders or on subjects specifically selected by lipid criteria were not used. Duplicate publications and publications with patient overlap were excluded. All identified publications were independently evaluated and selected by two investigators (S.M.B. and K.F.) for compliance with these criteria. The results were compared and disagreements were resolved by consensus. Considerable inconsistency exists concerning the nomenclature of the polymorphisms and alleles. The nomenclature used in this article is presented in Table 1.

Data extraction and analysis

Data were independently extracted and entered into separate databases by two investigators (S.M.B. and K.F.). The results were compared and disagreements were resolved by consensus.

All populations described in case-control studies were tested for Hardy-Weinberg equilibrium in cases and controls, and the genotype distributions between all groups were compared by χ^2 statistics. The relationships between genotype and outcome (either lipid parameter or risk of IHD) were analyzed with meta-analysis. Rare allele homozygotes were compared against wild-type homozygotes, in order to obtain maximal contrast. We did not consider a dominant model (comparing mutant homozygotes + heterozygotes versus wild-type individuals). Thus, heterozygotes were not taken into account in these meta-analyses. For lipid parameters, weighted mean differences (WMD) with corresponding 95% confidence intervals (95% CI) were calculated (Hedges and Olkin 1985). For dichotomous data on disease status, odds ratios (OR) with their corresponding 95% CI were calculated by using the fixed effects model according to Peto and Mantel-Haenszel (see Greenland and Rothman 1998). The raw data from each separately described population were entered as a separate stratum. Tests for heterogeneity were performed with each meta-analysis. Data were analyzed by using Review Manager version 4.1 (The Cochrane Collaboration 2000, <http://www.cochrane.org>).

Table 2 Characteristics of the individual included studies with IHD as outcome (*MI* myocardial infarction, *AP* angina pectoris, *CAD* by *CAG* coronary artery disease confirmed by coronary angiography, *DM* diabetes mellitus, *SP* signal peptide polymor-

phism). The numbers of cases and controls refer to the highest number analyzed for any of the polymorphisms. The numbers may vary between the polymorphisms

Reference	Ethnicity/ Nationality	Sex	Study population(s)	Polymorphism	Outcome
Benes et al. 2000b	Czech	Male	MI/AP by CAG	SP	IHD
Bohn et al. 1993, 1994	Norwegian	Both	MI	<i>Xba</i> I, SP	IHD
Corbo et al. 1997	Italian	Female	MI/AP	<i>Xba</i> I, <i>Eco</i> RI, SP	IHD
Gardemann et al. 1998	Caucasian	Male	CAD by CAG	SP	IHD
Hosking et al. 1992	Caucasian	Both	CAD by CAG	<i>Xba</i> I, <i>Eco</i> RI	IHD
Machado et al. 2001	Caucasian Brazilian	Both	CAD by CAG	<i>Xba</i> I, SP	IHD
Marshall et al. 1994	Caucasian	Both	CAD by CAG	<i>Xba</i> I, <i>Eco</i> RI, SP	IHD
Miettinen et al. 1994	Finnish	Both	MI/AP	<i>Xba</i> I	IHD
Moreel et al. 1992	French, Irish	Male	MI	<i>Xba</i> I, <i>Eco</i> RI	IHD
Nieminen et al. 1992	Finnish	Both	CAD by CAG	<i>Xba</i> I	IHD
Salazar et al. 2000	Caucasian	Female	CAD by CAG	<i>Xba</i> I, <i>Eco</i> RI, SP	IHD
Ukkola et al. 1993	Finnish	Both	CAD in DM	<i>Xba</i> I, <i>Eco</i> RI	IHD
Visvikis et al. 1993	French, Irish	Male	MI	SP	IHD
Wick et al. 1995	German	Both	CAD by CAG	<i>Eco</i> RI	IHD

Table 3 Characteristics of the individual included studies with lipid levels as outcome (MI myocardial infarction, TIA transient ischemic attack, AP angina pectoris, CAD by CAG coronary artery disease confirmed by coronary angiography, DM diabetes melli-

tus, SP signal peptide polymorphism). The numbers of IHD cases and IHD controls refer to the highest number analyzed for any of the lipid parameters. The numbers of individuals analyzed may vary between lipid parameters and the polymorphisms

Reference	Ethnicity/ Nationality	Sex	Study population(s)	Poly- morphism	Outcome
Aalto-Setälä et al. 1998	Finnish	Both	Ischemic stroke/TIA	<i>Xba</i> I	TC, LDL, TG
Abbey et al. 1995	Australian	Both	healthy volunteers	<i>Eco</i> RI	TC, LDL, TG
Benes et al. 2000a	Caucasian	Male	CAD by CAG	SP	TC, LDL, TG, apoB
Bohn et al. 1993	Norwegian	Both	MI, healthy controls	<i>Xba</i> I	TC, LDL, TG, apoB
Bohn et al. 1994	Norwegian	Both	MI, healthy controls	SP	TC, LDL, TG, apoB
Corbo et al. 1997	Italian	Female	MI/AP, healthy controls	<i>Xba</i> I, <i>Eco</i> RI, SP	TC, LDL, TG, ApoB
Deeb et al. 1992	Caucasian	Male	CAD by CAG, controls	<i>Xba</i> I	TC, LDL, apoB
Delghandi et al. 1999	Norwegian	Both	healthy controls	<i>Xba</i> I, <i>Eco</i> RI	TC, LDL, TG, apoB
Gaffney et al. 1993	Scottish	Both	healthy controls	SP	LDL, TG
Gardemann et al. 1998	Caucasian	Male	CAD, controls	SP	TC, TG, apoB
Glisic et al. 1997	Serbian	Both	healthy	SP	TC, TG
Hansen et al. 1993	Danish	Male	random volunteers	<i>Xba</i> I	TC, apoB
Hixson et al. 1992	Caucasian	Male	died of external causes	SP	TC
Iso et al. 1996	Caucasian	Both	healthy volunteers	<i>Eco</i> RI	TC, LDL, TG
Lopez-Miranda et al. 2000	Spanish	Male	ApoE3 homozygotes	<i>Xba</i> I	TC, LDL, TG, apoB
Lopez-Miranda et al. 1997	Spanish	Male	ApoE3 homozygotes	<i>Xba</i> I	TC, LDL, TG, apoB
Louhija et al. 1994	Finnish	Both	Centenarians	<i>Xba</i> I	TC, LDL, TG
Miettinen et al. 1994	Finnish	Both	MI/AP, healthy controls	<i>Xba</i> I	TC, LDL, TG
Moreel et al. 1992	French, Irish	Male	MI	<i>Xba</i> I, <i>Eco</i> RI	TC, LDL, TG, apoB
Pajukanta et al. 1996	Finnish	Both	healthy volunteers	<i>Xba</i> I, SP	TC, LDL, TG, apoB
Peacock et al. 1992	Swedish	Male	healthy controls	<i>Xba</i> I	LDL
Pouliot et al. 1994	Caucasian	Male	healthy	<i>Eco</i> RI	TC, LDL, TG, apoB
Series et al. 1993	Caucasian	Both	random subjects	<i>Eco</i> RI	TC
Turner et al. 1995	European	Both	offspring of MI patients, controls	<i>Xba</i> I, SP	TC, LDL, TG, apoB
Ukkola et al. 1993	Finnish	Both	DM	<i>Xba</i> I, <i>Eco</i> RI	TC, LDL, TG
Vilella et al. 1992	Spanish	Male	healthy volunteers	<i>Xba</i> I	TC, LDL, TG, apoB
Visvikis et al. 1993	French, Irish	Male	MI	SP	TC, LDL, TG, apoB

Results

Literature search

A total of 143 potentially relevant publications were identified. Of these, 45 were excluded because they were published before 1992. Twenty-one studies were excluded because the populations were non-Caucasian, or because the ethnicity of subjects could not be determined. Thirty-six publications were excluded because they had patient overlap with other publications, because patients were derived from a limited number of families, because they reported data affected by an intervention, because the reported data were not relevant for our analysis, or because subjects were selected on the basis of abnormal cholesterol levels (references available). One additional study selected patients on the basis of abnormal cholesterol levels but did provide potentially relevant data on the control group (Delghandi et al. 1999).

The remaining 40 publications reported potentially relevant data. Eleven of these provided a complete report of all data that were determined in the study (Benes et al. 2000b; Aalto-Setälä et al. 1998; Bohn et al. 1994; Gardemann et al. 1998; Louhija et al. 1994; Miettinen et al. 1994; Pajukanta et al. 1996; Salazar et al. 2000; Vilella et al. 1992; Visvikis et al. 1993; Wick et al. 1995). For the remaining 29 publications, the principal investigator was requested to provide data that were not shown in the article, and for 13 of these publications, additional data were provided (Abbey et al. 1995; Benes et al. 2000a; Bohn et al. 1993; Corbo et al. 1997; Delghandi et al. 1999; Hixson et al. 1992; Iso et al. 1996; Lopez-Miranda et al. 1997, 2000; Marshall et al. 1994; Moreel et al. 1992; Turner et al. 1995; Ukkola et al. 1993).

For the remaining 16 publications, the principal investigator could not be contacted, the requested data could not be retrieved, or permission from the local Medical Ethics Committee could not be obtained (references available). From these 16 publications, seven provided no otherwise relevant data, whereas from the remaining nine publications, the incomplete set of reported data was used (Deeb et al. 1992; Gaffney et al. 1993; Glisic et al. 1997; Hansen et al. 1993; Hosking et al. 1992; Machado et al. 2001; Nieminen et al. 1992; Peacock et al. 1992; Pouliot et al. 1994; Series et al. 1993). Thus, data from a total of 33 studies were used. The characteristics of these studies are summarized in Tables 2 and 3.

Genotype and allele distributions

The total population, which was analyzed for the *XbaI* polymorphism, was not in Hardy-Weinberg equilibrium, and this was attributed completely by the IHD cases ($\chi^2=6.786$, $df=1$, $P=0.009$), whereas all other populations and groups of cases and controls were in Hardy-Weinberg equilibrium ($\chi^2<1.8$, $df=1$, $P>0.18$). The *XbaI* genotypes distribution differed significantly between cases and controls ($\chi^2=9.915$, $df=2$, $P=0.007$). This was caused by a significantly lower *XbaI* X+ allele frequency among the cases with IHD (0.462 versus 0.494; $\chi^2=8.522$, $df=1$, $P=0.004$) that was mainly the result of a lower proportion of *XbaI* X+ homozygotes among the cases (0.198 versus 0.237; $\chi^2=8.930$, $df=1$, $P=0.003$). In the individual studies, no indications were found that cases and controls had different inclusion criteria with regards to (LDL) cholesterol and apoB levels. Moreover, a selection bias was very unlikely because *XbaI* had an unexpected inverse relation with

Table 4 Weighted mean differences for lipid parameters in rare allele homozygotes compared with common allele homozygotes. Data are weighted mean differences (WMD) and corresponding 95% CI for TC, LDL-C, triglycerides, and apoB, and the number

of individuals in each comparison. WMD quantifies the difference in concentrations between rare allele homozygotes (X+X+, E-E, DelDel, and common allele homozygotes X-X-, E+E+, InsIns)

Polymorphism	Lipid parameter	WMD (95%CI)	Rare allele		Common allele Homozygotes
			Homozygotes	Heterozygotes	
<i>XbaI</i>	Total cholesterol	0.15 (0.09–0.22) mmol/l, $P<0.0001$	X+X+	X+X–	X–X–
	LDL cholesterol	0.15 (0.09–0.20) mmol/l, $P<0.0001$	1139	2716	1583
	Triglycerides	0.04 (0.00–0.08) mmol/l, $P=0.07$	1036	2503	1480
	ApoB	0.06 (0.04–0.08) g/l, $P<0.0001$	917	2276	1339
Signal peptide			1010	2317	1291
			DelDel	InsDel	InsIns
	Total cholesterol	0.12 (0.06–0.18) mmol/l, $P<0.0001$	705	2910	2961
	LDL cholesterol	0.08 (0.03–0.13) mmol/l, $P=0.002$	443	1840	1754
			670	2808	2838
			613	2409	2611
<i>EcoRI</i>			E–E–	E–E+	E+E+
	Total cholesterol	–0.40 (–0.56–0.23) mmol/l, $p<0.0001$	42	450	865
	LDL cholesterol	–0.18 (–0.35–0.02) mmol/l, $p=0.03$	39	414	802
	Triglycerides	–0.07 (–0.28–0.13) mmol/l, $p=0.5$	39	421	805
			24	255	528
	ApoB	–0.05 (–0.15–0.05) g/l, $p=0.4$			

IHD (vide infra). The genotype ($\chi^2 < 3.1$, $df=2$, $P > 0.2$) and allele frequencies ($\chi^2 < 3.1$, $df=1$, $P > 0.08$) of the other two polymorphisms were not significantly different between cases and controls.

Homozygous genotypes and lipid parameters

Compared with wild-type (X–X–) individuals, *Xba*I X+ homozygotes had significantly higher TC and LDL-C levels: 0.15 mmol/l (95% CI=0.09–0.22; $P < 0.0001$) and 0.15 mmol/l (95% CI=0.09–0.20; $P < 0.0001$), respectively (Table 4). The apoB levels were higher in X+ homozygotes, than in wild-type individuals (0.06 g/l; 95% CI=0.04–0.08; $P < 0.0001$).

Homozygosity for the signal peptide deletion allele was associated with elevated TC levels (0.12 mmol/l; 95% CI=0.06–0.18; $p < 0.0001$) and LDL-C levels (0.09 mmol/l; 95% CI=0.03–0.13; $p = 0.002$), compared to wild-type (insertion allele) individuals. ApoB levels were indeed higher in deletion allele homozygotes (0.07 g/l; 95% CI=0.04–0.09; $p < 0.0001$).

Homozygosity for the rare *Eco*RI E– allele was associated with decreased levels of total cholesterol (0.40 mmol/l; 95% CI=0.23–0.56; $P < 0.0001$) and LDL-C (0.18 mmol/l; 95% CI=0.02–0.35; $P = 0.03$), compared with wild-type E+E+ individuals. The apoB levels were not associated with the E– allele in a recessive model. The triglyceride levels were not significantly associated with homozygosity for any of the three polymorphic sites.

Fig. 1 Odds ratios for individual studies and pooled odds ratios for the outcome ischemic heart disease in X+X+ homozygotes compared with X–X– homozygotes as the reference group. Thus, heterozygotes are not taken into account in this model. Point estimates and corresponding 95% CIs, and the absolute number of individuals are presented for individual studies, and for a pooled analysis. Square size is proportional to number of observations

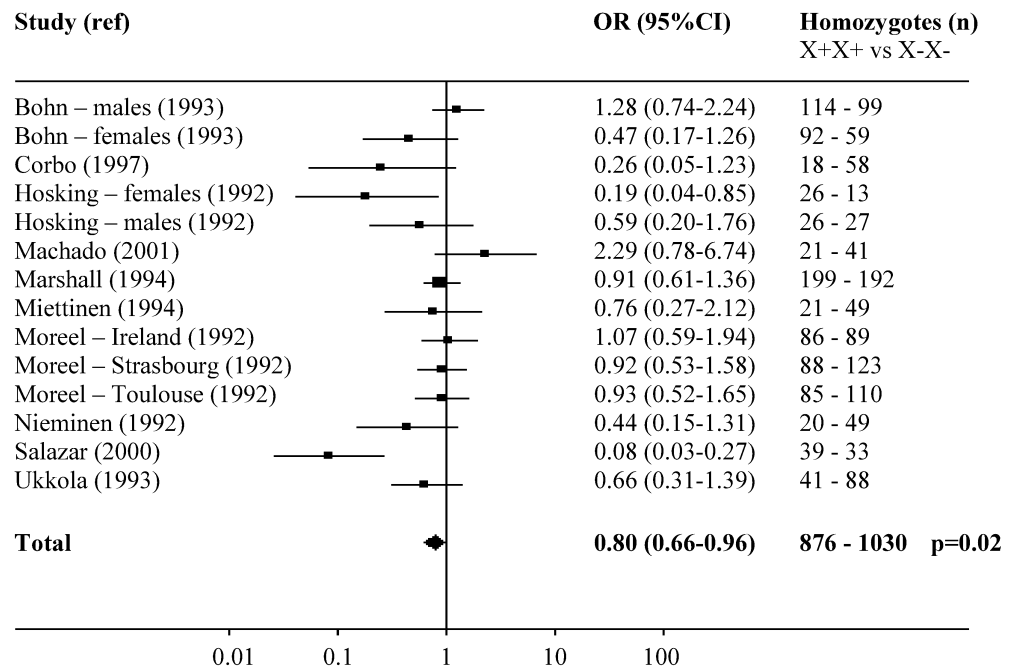


Fig. 2 Odds ratios for individual studies and pooled odds ratios for the outcome ischemic heart disease in deletion homozygotes compared with insertion homozygotes as the reference group. Thus, heterozygotes are not taken into account in this model. Point estimates and corresponding 95% CIs, and the absolute number of individuals are presented for individual studies, and for a pooled analysis. Square size is proportional to number of observations

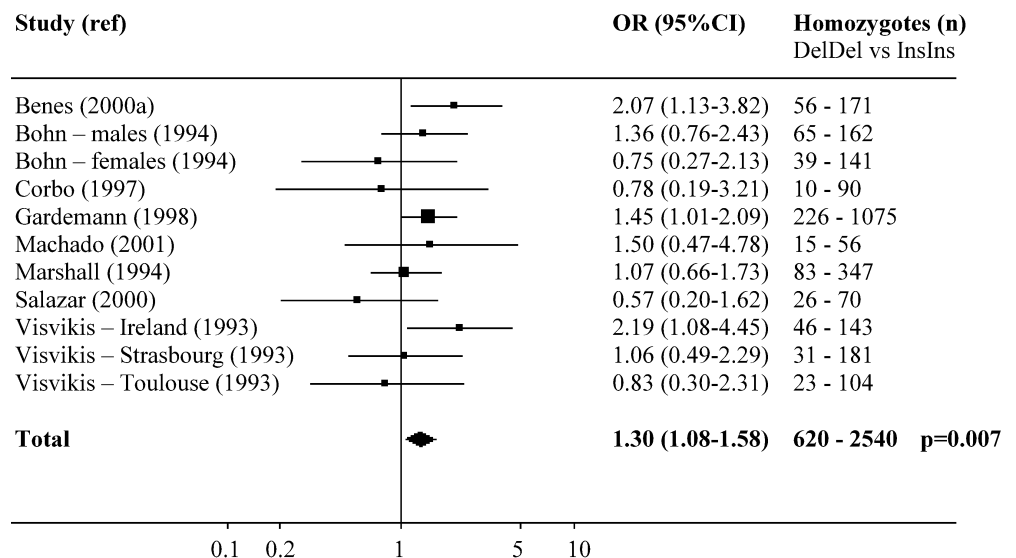
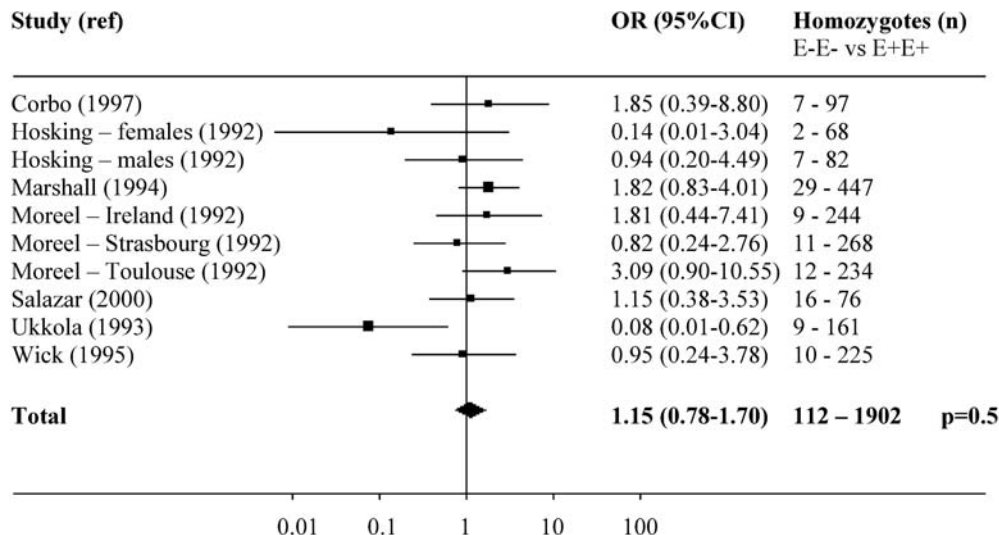


Fig. 3 Odds ratios for individual studies and pooled odds ratios for the outcome ischemic heart disease in E-E- homozygotes compared with E+E+ homozygotes as the reference group. Thus, heterozygotes are not taken into account in this model. Point estimates and corresponding 95% CIs, and the absolute number of individuals are presented for individual studies, and for a pooled analysis. Square size is proportional to number of observations



Genotype and IHD

In addition to the comparison of genotype distributions (rare allele homozygotes, heterozygotes, common allele homozygotes) between IHD cases and controls, we quantified the risk of IHD associated with homozygosity for the rare allele, as compared with homozygosity for the common allele. Homozygosity for the *Xba*I X+ allele was associated with a reduced risk of IHD (OR=0.80; 95% CI=0.66–0.96; $P=0.02$; Fig. 1). An analysis restricted to populations whose lipid profiles were available showed similar results (data not shown). Homozygosity for the signal peptide deletion allele was associated with an increased risk of IHD (OR=1.30; 95% CI=1.08–1.58; $P=0.007$; Fig. 2). The *Eco*RI polymorphism was not significantly associated with IHD (OR=1.15; 95% CI=0.78–1.70; $P=0.5$; Fig. 3).

Discussion

In the present meta-analysis, variation at the APOB gene locus exerted differential effects on LDL-C and apoB levels and on IHD risk. In the general population, high levels of apoB lead to an increased risk of IHD (Walldius et al. 2001), and in patients on statin therapy, apoB levels have recently been proposed as a more accurate therapy guidant than LDL-C (Miremadi et al. 2002; Sniderman et al. 2003). The findings of the present study suggest that these relationships may not have a simple molecular basis in the APOB gene.

The *Xba*I X+ allele was significantly associated with higher LDL-C and apoB levels. Strikingly, the *Xba*I X+ allele was also associated with a significantly decreased risk of IHD. ApoB acts as ligand for LDL-receptor-mediated uptake of LDL in the liver. Among patients with a mutated LDL receptor, large variation has been observed in the severity of hypercholesterolemia (Sijbrands et al. 2001). The clinical consequences of mutated LDL receptors depend on interactions with unknown additional risk

factors (Sijbrands et al. 2000). However, disorders of apoB itself could change the atherogenic properties of LDL without an influence on such additional factors. The inverse associations of the polymorphic *Xba*I site with hypercholesterolemia and IHD risk could be explained by structural changes of apoB that concomitantly cause hypercholesterolemia and modify LDL toward a less atherogenic particle. ApoB has arginines positioned in the carboxy terminal part of the protein; this results in a receptor-binding site at amino acid residues 3359–3369 (Boren et al. 2001). An interaction between arginine and tryptophan is essential for the correct refolding of apoB during hydrolysis of very low density lipoproteins (VLDL) to LDL. The absence of an arginine leads to reduced affinity for the LDL receptor and may also change the refolding of apoB during the intravascular remodeling of VLDL to LDL (Boren et al. 2001). Alternatively, a structural disorder may affect the interaction site of apoB with proteoglycans of the extracellular matrix (Skalen et al. 2002). Theoretically, such structural changes of apoB could result in accumulation of less atherogenic LDL in the circulation. The polymorphic *Xba*I site is located in exon 26 and does not cause an amino acid substitution. Therefore, the observed associations probably result from co-segregation with one or more functional variants in the APOB gene or a gene located nearby. Such linkage disequilibrium might exist with variants that affect the LDL-receptor-binding region of apoB and refolding of the protein (Dunning et al. 1993; Umans-Eckenhansen et al. 2002).

The signal peptide deletion allele of apoB is also associated with hypercholesterolemia and with an increased risk of IHD. This deletion allele results in an absence of three amino acids, which could lead to diminished hydrophobicity of the signal peptide, and this could improve the translocation of apoB from the cytoplasm leading to an increased secretion rate of apoB-containing lipoproteins (Randall and Hardy 1989). To our knowledge, a comparison of LDL properties between *Xba*I X+ homozygotes and deletion allele homozygotes has not been performed.

Despite a significant and relatively large LDL-C-lowering effect of the *EcoRI* polymorphism, no effect on the risk of IHD was observed. The *EcoRI* polymorphism predicts a Glu-Lys substitution in exon 29 of the APOB gene. This mutation may alter the tertiary structure of apoB, thereby affecting the interaction with the LDL receptor.

Design and limitations

Several issues have to be taken into account when interpreting the present meta-analysis. A major weakness of any meta-analysis is publication bias, which results from not publishing "non-significant" data (Ioannidis et al. 2001). An ideal meta-analysis should contain all data from positive and negative studies, but this is impossible because many investigators of unpublished studies remain anonymous, have moved or retired, or are unwilling or unable to provide unpublished data. (Campbell et al. 2002). Because this is particularly true for older studies, we restricted our analysis to the last 10 years. The strength of our analysis lies in the fact that we collected a substantial amount of unpublished data from this recent time span. Consequently, our findings clearly do not incorporate a trend toward selection of positive findings. More importantly, it results in analyses that are not influenced by calendar time, which can have a strong influence on the relationship between genotype and disease (Sijbrands et al. 2001). For example, a finding in a large pedigree with hypercholesterolemia suggests that meta-analyses into the relationship between genotypes and chronic disease should be adjusted for calendar time or should be restricted to present-day patients. Another problem relating to meta-analysis in general is the use of different outcome definitions in the individual studies. However, the methods were identical within each individual study included here, and therefore the inconsistencies could not have introduced an important systematic bias. Moreover, some of the discrepancies between individual studies could be caused by differences in ethnic background or lifestyle, and therefore we restricted our analyses to Caucasian populations. Finally, a particular difficulty may have been introduced by the fact that that we analyzed the relationship between APOB genotypes and intermediate traits (LDL-C and apoB) and the risk of IHD. Such a line of investigation suggests analyses of a causal pathway, but these intermediate traits and clinical endpoints were in part analyzed in different studies. However, similar results were obtained in additional analyses restricted to studies that provided information on lipids and IHD.

Interpretation

The three most frequently analyzed polymorphisms in the APOB gene had differential effects on lipid profile and IHD risk. Hypercholesterolemia associated with polymorphisms in the APOB gene does not necessarily lead to an increased risk of IHD. Future research is needed to assess

whether or not the specific polymorphisms influence the atherogenic properties of the LDL particles. Moreover, the effect should be analyzed regarding linkage disequilibrium between the polymorphisms. Nonetheless, we conclude that only the signal peptide deletion allele contributes to an increased risk of IHD that is associated with hypercholesterolemia and high apoB levels. However, the findings from the other two frequent polymorphisms (*XbaI* and *EcoRI*) emphasize that the relationship between IHD and hypercholesterolemia and high apoB levels in the general population is complex and involves more than variation in the APOB gene.

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