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Apolipoprotein E polymorphisms and type 2 diabetes: A meta-analysis of 30 studies including 5423 cases and 8197 controls

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a highly complicated metabolic disorder for which there is worldwide effort for the identification of susceptibility genes. Polymorphisms of the Apolipoprotein E (ApoE) gene are associated with plasma lipid and lipoprotein levels and influence cardiovascular risk. Since insulin resistance is known to be strongly associated with metabolic dyslipidemia, ApoE polymorphisms have been implicated in predisposition to diabetes but the results of the individual studies were inconclusive. We present here a meta-analysis of population-based case–control genetic-association studies relating ApoE polymorphisms and T2DM. We included in the analysis 30 studies, which reported data of ApoE genotypes in 5423 T2DM patients and 8197 healthy unrelated controls. Multivariate and univariate methods suggest a significant role played by the E2 allele, since carriers of the E2 allele were at elevated risk for T2DM (Odds Ratio = 1.18, 95% CI: 1.02, 1.35). There was no evidence for publication bias or other small-study related bias or significant heterogeneity in the analyses. Cumulative meta-analysis revealed no trend of the effect estimates over time and influential analysis excluded the possibility of a single influential study. E2 allele of ApoE seems to be a moderate risk factor for T2DM. Meta-regression analysis provided some weak evidence that the risk conferred by E2 allele is mediated through altering serum lipid levels (Total Cholesterol, LDL and HDL). Further studies are needed in order to elucidate the metabolic mechanism of this association as well as to study its effects on larger populations.

Introduction

Type 2 diabetes mellitus (T2DM; formerly known as non-insulin dependent diabetes mellitus, NIDDM) is a complicated metabolic disorder. The incidence of diabetes is dramatically increasing worldwide due to changes in human behavior and nutrition, since sedentary lifestyle and obesity are important risk factors for the development of T2DM [1]. The disease is epidemic and is believed that in 2010 two hundred twenty-one million people will suffer from diabetes worldwide, an increase of 46% compared to 2000 [2]. Although T2DM is considered to be an adults' disease, there is epidemiologic evidence of increasing incidences on younger people [3]. Due to the increase of diabetes incidence and the expansion of the disease on youth, great effort has been put on identifying susceptibility genes for the disease [4–9].

Apolipoprotein E (ApoE) is a candidate gene for the development of T2DM due to its critical role in the lipid metabolism. ApoE is mapped at chromosome 19 and is a polymorphic gene, possessing three major alleles (E2, E3, E4) with six possible genotypes (E2/2, E2/3, E2/4, E3/3, E3/4, E4/4). The gene encodes a protein of 299 amino acids with three isoforms (ε2, ε3, ε4) which differ in two amino acid residues at positions 112 and 158. The ApoE ε3 isoform possesses a cysteine at position 112 and an arginine at position 158, while ε2 possesses cysteines at both positions and ε4 possesses arginines at both positions [10]. The most common isoform is ε3 with a frequency of approximately 70–80%. The other two isoforms, ε2 (~5–10%) and ε4 (~10–15%), have been thought to be dysfunctional [11].

Human lipoproteins are found in plasma and are composed of a nonpolar lipid core, consisting of triglycerides and cholesteryl esters and an external layer of phospholipids and apolipoproteins [12]. Apolipoproteins are the only protein components of this complex. There are about a dozen of different apolipoproteins represented by five major types (A, B, C, D, E) some of which are categorized in other subtypes [13]. ApoE is synthesized mainly in the liver but it is also found in other tissues like brain, spleen and kidneys. Similar to the other apolipoproteins, ApoE plays a role in the stability and solubility of lipoproteins during their circulation in human plasma. ApoE is important for the development of several plasma-lipoprotein lipid particles like very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), high

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density lipoproteins (HDL) and chylomicrons. Besides its role to the formation of the different kind of lipoproteins, ApoE acts also as a ligand for the binding of lipoproteins to plasma lipoproteins receptors [14]. Low-density lipoprotein receptor (LDLR) is a membrane protein that mediates the endocytosis of cholesterol-rich LDL and specifically recognizes Apolipoprotein E. Thus, it plays an important role in the regulation of plasma and cellular lipid concentrations [15].

Apolipoprotein E plays a significant role in lipid formation and thus, it has been found to be associated with plasma lipid and lipoprotein levels [16,17]. The three isoforms have different chemical stability ($\varepsilon4 < \varepsilon3 < \varepsilon2$) [18]. Moreover, the genetic variation of Apolipoprotein E plays an important role in dietary fat clearance on the metabolism of dietary fats [19]. Several large meta-analyses have documented that E4 is associated with increased risk for Coronary Heart Disease (CHD) [20,21], whereas the E4 allele is also significantly associated with Ischemic Cerebrovascular Disease (ICD) [22,23]. In a recent large meta-analysis, individuals carrying the E2/2 genotype had about 31% lower mean low-density lipoprotein (LDL) than those with the E4/4 genotype. Compared to individuals with the E3/3 genotype, E2 carriers had a 20% lower risk of CHD and E4 carriers have a slightly higher risk [23]. In addition to the cardiovascular risk, E4 allele has been found to represent a major risk factor for Alzheimer's disease [24] and a risk factor for dementia in Parkinson disease [25], whereas on the other hand, it has been shown to confer protective effect of up to 40% in age-related macular degeneration [26]. Another meta-analysis suggested that E4 allele affects cognitive performance in healthy aging, although the influence is relatively small and specific to certain domains of cognitive performance [27]. Insulin resistance is known to be strongly associated with metabolic dyslipidemia and the correlation of lipid profiles with diabetic phenotypes is important, since T2DM patients have an atherogenic lipid profile, which greatly increases their risk of CHD compared to people without diabetes [28,29]. Consequently, ApoE polymorphisms have also been implicated in predisposition to diabetes, but the results of the individual studies were inconclusive. We present here for the first time in the literature a meta-analysis of population-based case–control genetic-association studies relating Apolipoprotein E polymorphisms and T2DM.

Materials and methods

Retrieval of published studies

We performed a systematic computerized literature search using PUBMED for papers published before October 1st, 2008. The search was performed using various combinations of keywords like (''ApoE" OR ''Apolipoprotein E") AND (''polymorphism" OR ''variant" OR ''allele" OR ''mutant" OR ''mutation") AND (''type 2 diabetes" OR ''NIDDM" OR ''type II diabetes" OR ''diabetes type 2" OR ''diabetes type II" OR ''non-insulin dependent diabetes"). We also retrieved related articles from the reference lists of the papers that we had identified during the search. The full text of the articles was read in order to decide if the article included data of interest. We also checked for special meeting issues in order to retrieve studies that were not included in computer indices and may bias the meta-analysis results if not included [30]. We also decided to include in our meta-analysis, studies written in languages different than English in order to avoid local literature bias [31]. No study was rejected because of low quality data and no quality scoring was performed since modern approaches advocate against this approach [32,33].

Data extraction

The full text of the retrieved articles was read in order to find the data of the genotypes for diseased (cases) and healthy individuals (controls). Some of the studies reported incomplete genotype data and we had to calculate them using other information in the manuscripts such as allele frequencies etc. Studies from which it was impossible to retrieve any useful data for diseased and healthy individuals like genotypes or allele carriers that could be potentially used in any genotype or allele contrast in the subsequent analyses (see below) were rejected. From each study we extracted the following data: PUBMED ID, first author's name, year of publication, ethnicity and country of population studied and population's racial descent. For every study we retrieved the number of healthy and diseased individuals for each polymorphism and we calculated the ones that were not reported. We also collected summary study-level data for cases and controls concerning the total cholesterol (TC) levels, the low-density lipoproteins (LDL), the high density lipoproteins (HDL) and the triglyceride (TG) levels that could be potentially used in a meta-regression analysis [34].

Statistical analysis

The Odds Ratio (OR) was used to compare contrasts of genotypes and alleles between cases and controls. In case of zero cell counts a continuity correction was applied by adding 0.5 to all cells of the contingency table. Initially, for avoiding multiple comparisons comparing the effect of the genotypes against a reference genotype, we used a recently proposed multivariate random-effects method of meta-analysis that takes into account the pairwise correlations of the ORs [35]. Genotype E3/3 was chosen as reference category (baseline) for this analysis since it is the most common among the healthy and diseased subjects with a frequency of about 67% as well as because the literature suggests that this is the ''wild-type" genotype (i.e. the $\varepsilon4$ isoform has been thought to be dysfunctional). Afterward, we proceeded by grouping the genotypes and allele carriers in order to derive a summary OR for the most likely genetic model of inheritance. Data were combined using random-effects models [36] with inverse-variance weights. In case of heterogeneity, random-effects models are more appropriate since they estimate a between-study variance (τ^2), whereas when heterogeneity is absent, random- and fixed-effects methods coincide. We calculated combined ORs along with their 95% CIs for each genotype or allele contrast (i.e. E2 carriers vs. the others, E2 alleles vs. the others and so on) using a standard random-effects method [36]. The between-study heterogeneity was evaluated using the chi-square based Cochran's Q statistic [37] and the inconsistency index (I^2) [38].

Publication bias or other small study bias was evaluated using the rank correlation method of Begg and Mazumdar [39]. We also used the fixed-effects regression method of Egger et al. [40] and its random-effects analog [41]. In an attempt to identify potential influential studies, we calculated the effects estimates (ORs) by removing an individual study each time and then checked if the overall significance of the estimate or of the heterogeneity statistic was altered. Cumulative meta-analysis [42,43] was performed in order to identify a possible trend of the combined estimate over years, a situation that often introduces a special form of bias (the so-called ''Proteus phenomenon" or the ''winner's curse") in genetic-association studies [44]. For the detection of the time-trend, we used the standard cumulative meta-analysis approach [42], which consists of visually inspecting the plot and a recently proposed regression-based method [45]. We performed tests for the Hardy–Weinberg Equilibrium (HWE) at the genotype distribution of the controls' population, in order to assess the influence of the departures from HWE on the overall estimates [46]. For testing HWE given that we have to deal with multiple alleles, we used a specialized method along with the accompanied software (http://www.biology.ualberta.ca/jbrzusto/hwenj.html) [47]. Subgroup analyses were conducted appropriately in order to

investigate the effect of dichotomous variables (racial descent of the populations, deviations from HWE, etc.), whereas meta-regression analysis was carried out concerning continuous variables (LDL, HDL, TG, TC). For comparing the mean levels of covariates between cases and controls we used a standard *t*-test. For the statistical analysis we used Stata 10 (Stata Corporation, College Station, TX, USA). In all cases statistically significant results were declared those with p-value < 0.05.

Results

The literature search through PUBMED yielded initially 133 published articles. We performed a screening on the identified articles to choose those which include valuable data for our purposes. One hundred and three papers were excluded since they contained no useful information. For instance, they reported in the abstract some of the search terms (i.e. diabetes and ApoE) but there were actually studies that had nothing to do with diabetes. Few studies were discarded also because they did not report any data that can be used in the analysis (i.e. the genotype or allele frequencies for any of the contrasts between cases and controls). Usually, these were studies in which diabetes was not among the primary outcomes. Finally, we came up that 30 published research studies reported data for a healthy (non-diabetic) group and a T2DM patients group. The identified studies contained in total information for 8197 healthy (non-diabetic) subjects and 5423 T2DM patients. None of the identified studies used a family-based design. We found 15 studies on Asian-descent populations, 11 studies on Caucasian or European-descent populations, whereas five studies reported data concerning populations of mixed origin (Caucasian/African-American or Caucasian/Native-American). One study [48] contained data for two distinct populations (Caucasians and Hispanics) and thus, it was included in the meta-analysis as two independent studies. The information concerning all included studies (first author, year of publication, country, racial descent of the population and sample size of cases and control for each genotype) is presented in Table 1. Three studies were written in languages other than English (two in Chinese and one in Spanish) [49–51] and these were retrieved, translated and included in the analysis in order to avoid the local literature bias [31].

The multivariate random-effects method [35] yielded a statistically significant OR equal to 1.17 for the contrast of E2/3 genotype compared to the wild type E3/3 (95% CI 1.00–1.36, p-value = 0.049). The ORs for the other E2-carriers genotypes (E2/2 and E2/4) compared to E3/3 were also found to be of similar magnitude even though did not reach statistical significance (Fig. 1). This should be attributed mainly to the small sample sizes of these groups. We then, proceeded by collapsing the genotypes and performing traditional univariate meta-analyses using random-effects methods. The contrast of the E2 carriers vs. non-carriers yielded a significant estimate (OR = 1.18, CI 1.02–1.35, p-value = 0.023). The allele-based contrasts revealed also a statistically significant OR for the contrast of E2 allele vs. the others (OR = 1.17, CI 1.03–1.33, p-value = 0.020). These results are presented in the forest plots of Figs. 2 and 3, respectively. Thus, it is reasonable to assume that the E2 allele is an independent risk factor for the development of T2DM.

In all of the analyses reported above, the heterogeneity was low (I^2 < 25%, p-value for heterogeneity > 0.15 in all cases), strengthening our beliefs concerning the validity of the results. Subgroup analysis comparing the estimates found in different ethnic groups revealed also no significant differences (p-values > 0.3 in all cases).

Table 1
Characteristics of the studies included in the meta-analysis. We list the first author and the year of publication, the country and the racial descent (Asian, Caucasian and other; Other usually refer to mixed populations, e.g. Caucasians/Africans or Caucasians/native Americans) of the population and the genotypes for cases and controls.

Author	Year	Country	Descent	Cases							Controls						
				E2/2	E2/3	E2/4	E3/3	E3/4	E4/4	Total	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4	Total
Eto [60]	1986	Japan	Asian	0	9	0	73	21	2	105	1	10	1	80	16	3	111
Eto [53]	1987	Japan	Asian	0	5	1	150	50	5	211	0	1	0	42	14	2	59
Vogelberg [83]	1988	Germany	Caucasian	3	2	1	26	4	2	38	10	124	15	617	236	29	1031
del Pozo [49]	1988	Spain	Caucasian	0	6	0	27	9	0	42	0	20	0	76	20	0	116
Imari [84]	1988	Japan	Asian	0	12	2	63	17	0	94	0	8	0	66	17	0	91
Shriver [85]	1991	USA	Other	0	19	2	187	44	2	254	2	64	7	711	169	11	964
Boemi [86]	1993	Italy	Caucasian	4	56	6	315	52	3	436	3	43	3	257	51	7	364
Horita [61]	1994	Japan	Asian	3	27	3	317	95	10	455	2	35	4	414	111	10	576
Eto [59]	1995	Japan	Asian	1	25	1	192	55	7	281	2	35	4	414	111	10	576
Kamboh [48]	1995	USA	Caucasian	0	23	5	62	26	0	116	6	88	19	382	150	14	659
Kamboh [48]	1995	USA	Other	0	28	2	150	50	5	235	4	29	5	332	74	2	446
Vauhkonen [87]	1997	Finland	Caucasian	0	7	3	48	20	8	86	0	9	2	76	33	5	125
Kimura [88]	1998	Japan	Asian	0	13	4	125	34	1	177	0	25	0	181	42	3	251
Guangda [54]	1999	China	Asian	1	20	1	109	31	4	166	1	7	2	53	7	2	72
Inamdar [56]	2000	India	Asian	2	8	3	17	16	14	60	1	9	2	10	8	10	40
Kalix [57]	2001	Switzerland	Caucasian	1	21	0	136	36	2	196	0	37	0	205	50	0	292
Hsieh [55]	2002	Taiwan	Asian	1	19	16	252	20	6	314	0	4	1	126	13	6	150
Santos [89]	2002	Mexico	Other	0	0	0	32	3	1	36	1	2	1	10	8	0	22
Zhang [51]	2003	China	Asian	0	5	1	55	12	1	74	1	23	1	134	31	1	191
Xiang [50]	2003	China	Asian	4	30	2	161	50	8	255	1	10	1	75	17	3	107
Liu [90]	2003	China	Asian	1	47	3	193	53	1	298	0	4	2	64	11	0	81
Powell [91]	2003	UK	Caucasian	4	41	4	210	50	7	316	2	7	1	57	21	0	88
Duman [92]	2004	Turkey	Caucasian	1	11	2	81	13	4	112	0	12	3	62	16	1	94
Camsari [52]	2005	Turkey	Caucasian	9	19	5	63	21	7	124	14	27	9	97	19	5	171
Leiva [93]	2005	Chile	Other	0	12	4	133	43	1	193	0	10	3	87	39	0	139
Errera [94]	2006	Brazil	Other	0	13	2	68	12	0	95	0	7	0	77	23	0	107
Morbois Trabut [95]	2006	France	Caucasian	2	31	1	143	33	0	210	5	71	14	294	87	10	481
Singh [96]	2006	India	Asian	1	4	2	78	5	0	90	1	7	0	74	13	2	97
Ilhan [97]	2007	Turkey	Caucasian	4	9	0	77	18	0	108	0	4	0	40	2	0	46
Kwon [98]	2007	Korea	Asian	0	13	3	63	14	1	94	0	5	0	70	12	1	88
Vaisi-Raygani [58]	2007	Iran	Asian	2	29	2	77	35	7	152	1	86	2	381	83	9	562
			Total	44	564	81	3683	942	109	5423	58	823	102	5564	1504	146	8197
			%	0.8	10.5	1.5	67.9	17.3	2	100	0.7	10	1.2	68	18.3	1.8	100

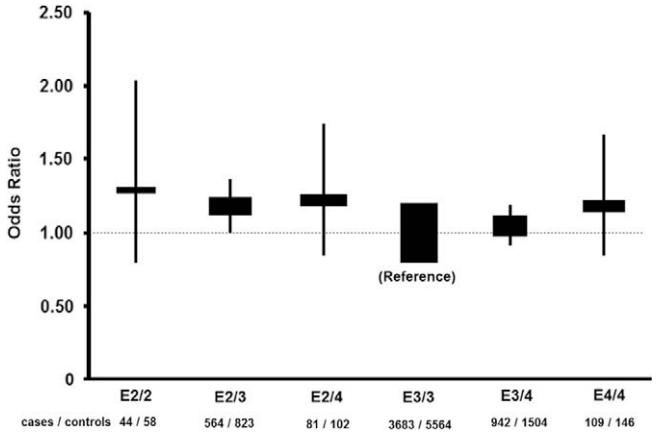

Fig. 1. Graphical representation of the results of the multivariate meta-analysis concerning the comparison of the five genotypes to the wild type genotype E3/3 using the method proposed in [35]. The size of each symbol that represents an Odds Ratio is inversely proportional to the variance of the corresponding log Odds Ratio. Vertical lines represent the 95% CI.

Using Begg's test, the regression-based tests as well as by visual inspection of the funnel plots (i.e. Fig. 4 for the E2 carriers) we found no evidence for publication or other small-study-related bias (p-values were >0.7 in most of the cases). The inspection of the cumulative meta-analysis plots (Fig. 5 for the E2 carriers) showed no evidence for trend of the effect estimates over time and the same conclusions were drawn from the formal regression-based statistical tests [45]. The analysis for deviations from HWE revealed that the control groups of seven studies [52–58] and the Hispanic population [48], deviated significantly from HWE. However, subgroup analysis comparing the studies that are on HWE vs. the studies that deviate, revealed that the overall estimates did not differ significantly (p-value = 0.932 for the E2 allele comparison and p-value = 0.749 for the E2 carriers comparison). Furthermore, studies that deviate from HWE did not differ significantly (i.e. $p > 0.4$ in all situations) from those that did not, in a number of measurable characteristics (year of publication, racial descent of the included populations, minor allele frequency or total sample size). Thus, HWE should not be considered as a factor influencing the overall results.

The influential analysis revealed that no single study was responsible for the overall significance of the estimates. After removing each study and re-calculating the combined estimates, in both the E2 allele comparison and the E2 carriers comparison the overall estimates as well as their significance remained nearly unchanged. Four of the included studies [53,59–61], were performed by the same research group and in the materials and methods sections of the respective manuscripts, there was no evidence whether the studies contained overlapping sets of individuals or not. By excluding the four studies altogether and performing the whole meta-analysis again for the E2 allele, the overall estimate as well as its significance remained nearly unchanged (OR = 1.19, CI 1.01–1.40, p-value = 0.028). We also performed a separate analysis excluding the five studies performed on mixed populations since they could be sources of population stratification bias. Once again the magnitude of the association and the statistical significance were not altered (OR = 1.16, CI 1.01–1.33, p-value = 0.032 CI). Thus, in any case the overall conclusions drawn from our meta-analysis remain unaffected.

Although not all studies reported separately summary data for cases and controls concerning the biochemical parameters (TG, TC, LDL and HDL), some useful insights could be obtained. For

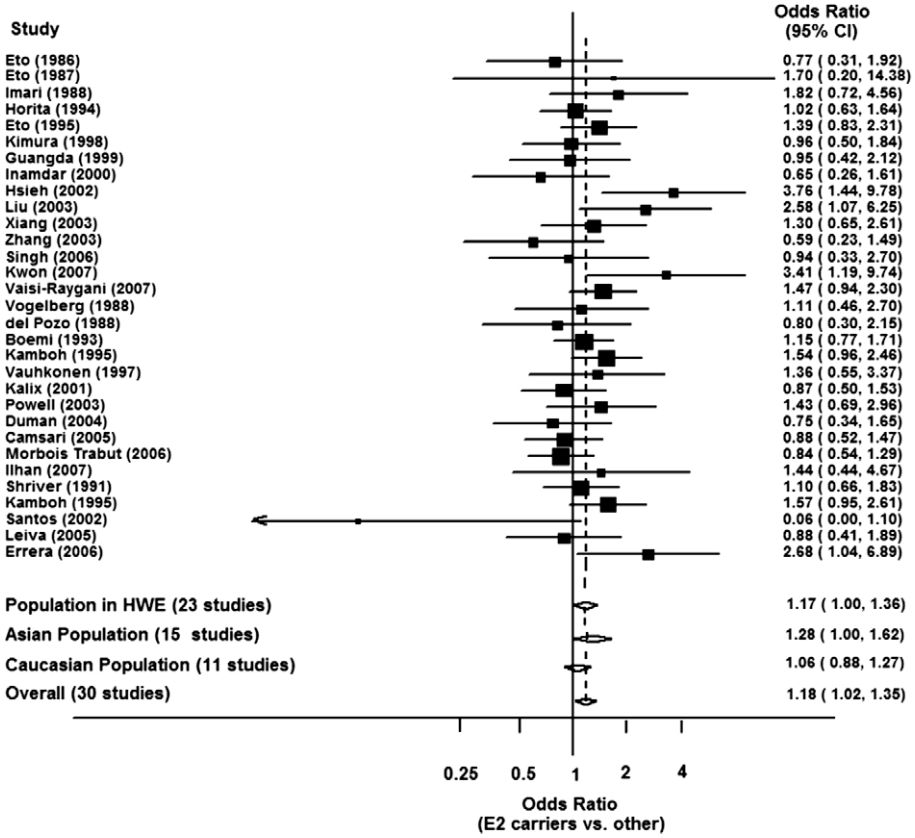

Fig. 2. Forest plot for the results of the meta-analysis of E2 carriers compared to the other genotypes. The random-effects method of DerSimonian and Laird was used with inverse-variance weights. The size of each symbol that represents a log Odds Ratio is inversely proportional to its variance. Subgroup analyses of various ethnic groups as well as for studies that were found in HWE are presented also. Horizontal lines represent the 95% CI for each study.

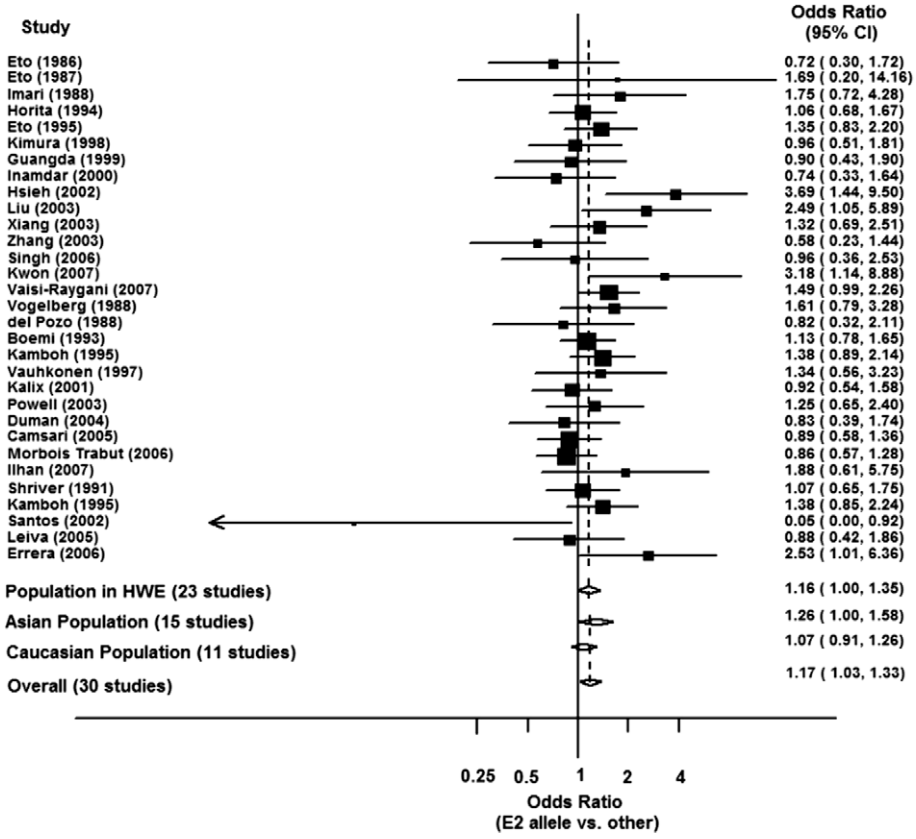

Fig. 3. Forest plot for the results of the meta-analysis of E2 allele compared to the other alleles. The random-effects method of DerSimonian and Laird was used with inverse-variance weights. The size of each symbol that represents a log Odds Ratio is inversely proportional to its variance. Subgroup analyses of various ethnic groups as well as for studies that were found in HWE are presented also. Horizontal lines represent the 95% CI for each study.

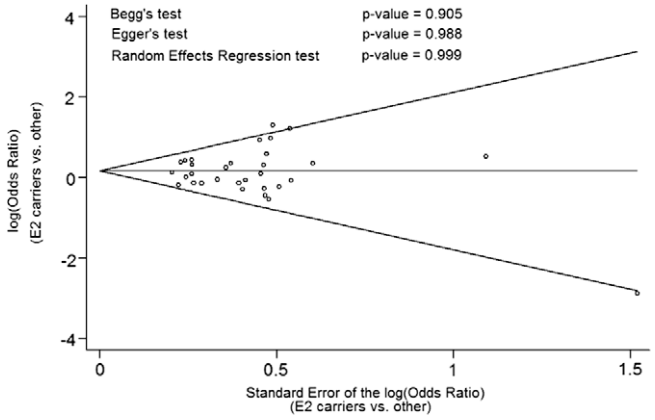

Fig. 4. Funnel plot for the results of the meta-analysis of E2 carriers compared to the other genotypes. Asymmetry of the plot indicates publication or other small studies related bias. The results of the three formal tests for detecting such bias are listed.

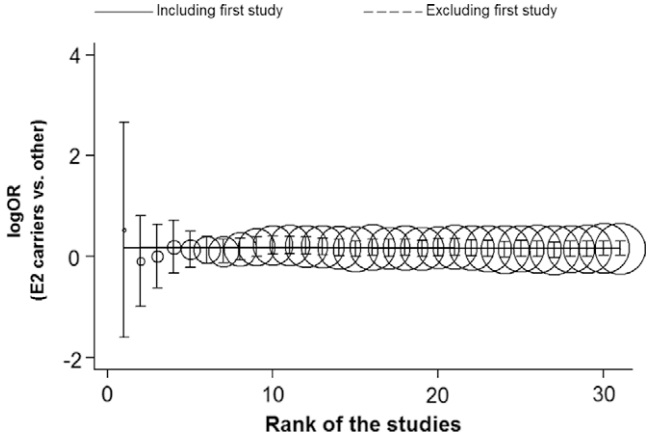

Fig. 5. Cumulative meta-analysis plot for the results of the meta-analysis of E2 carriers compared to the other genotypes. The studies are sorted by year of publication. A slope significantly different from zero indicates time-trend related bias. The regression-based test [45] indicated no such bias. Vertical lines represent the 95% CI. The two regression lines, excluding the first study and including all studies, nearly coincide.

instance, TC levels in cases were higher compared to controls (215 vs. 191 mg/dL, p-value = 0.015 based on 17 studies), TG were also elevated (209 vs. 129 mg/dL, p-value = 0.003 based on 16 studies) whereas HDL was, as expected, elevated in controls (43 vs. 48 mg/dL, p-value = 0.001 based on 13 studies). The difference in LDL levels was smaller in magnitude and did not reach nominal statistical significance (130 vs. 121 mg/dL, p-value = 0.069 based on 12 studies). These results are in agreement with previous estimates [28,29], even though in our case are based on a large number of subjects. Using these study-level variables (for cases and controls, respectively) as covariates in a meta-regression analysis [34] we failed to find a statistically significant effect (Fig. 6). However, there was some weak evidence that elevated TC and LDL levels in controls are associated with reduced risk attributed to E2 allele ($\beta = -0.0036$, $p = 0.28$ and $\beta = -0.0037$, $p = 0.27$, respectively), whereas increased HDL levels in controls are associated with increased risk of the E2 allele ($\beta = 0.022$, $p = 0.18$).

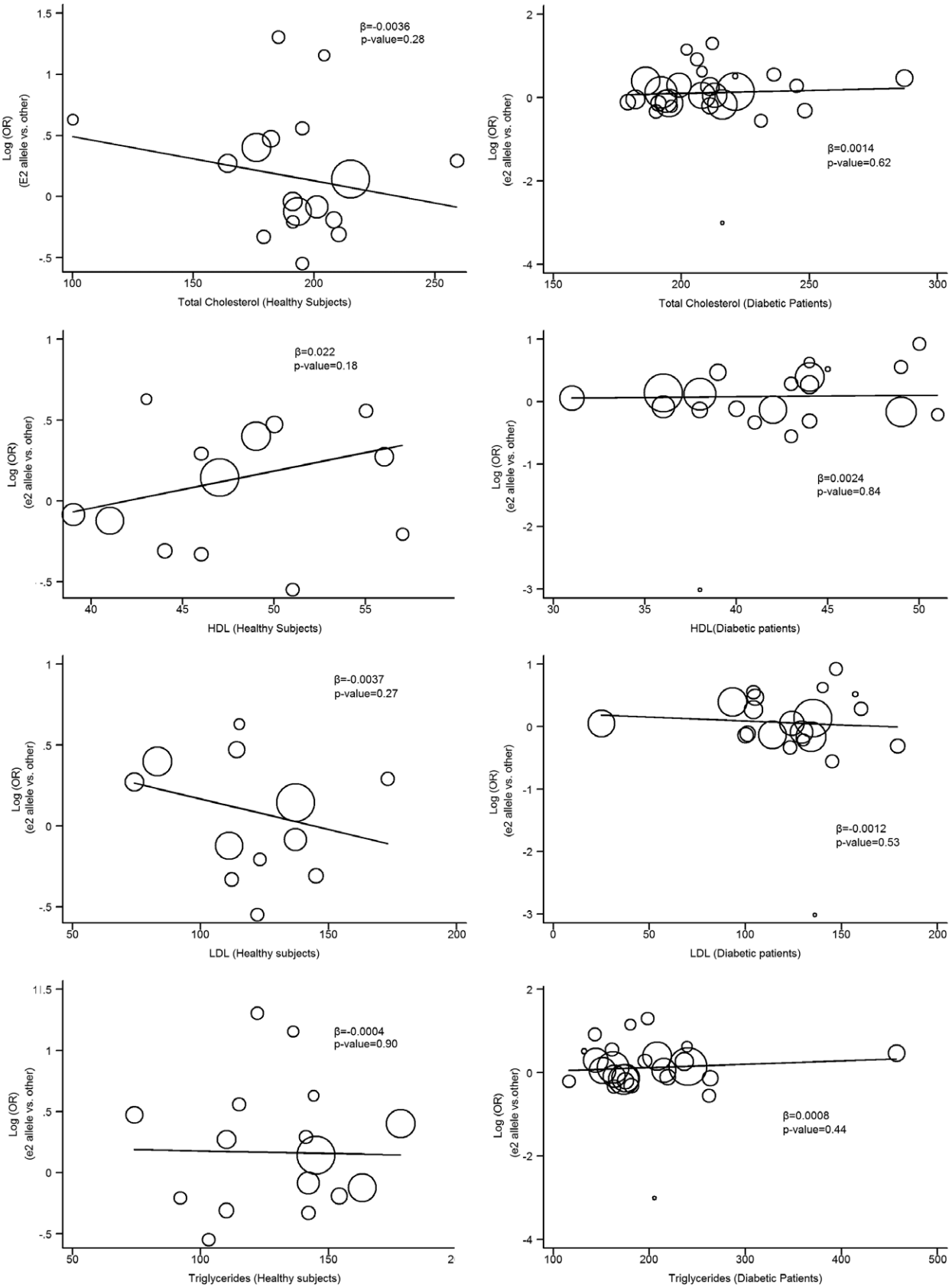

Fig. 6. The logOR for the contrast of E2 allele vs. others, plotted against Total Cholesterol, HDL, LDL and Triglyceride levels in controls in the left panel, and diabetic individuals in the right panel. The size of each symbol that represents a log Odds Ratio is inversely proportional to its variance. We also list the coefficient obtained from the meta-regression and the associated *p*-value.

Discussion

To our knowledge this is the first meta-analysis which investigates the association of Apolipoprotein E polymorphisms with T2DM. Meta-analysis is a methodology suitable for dealing with genetic-association studies concerning common, low penetrant variants, since in the majority of the published cases, the risk associated with a particular variant has been shown to be in the range between 1.1 and 1.5 [62,63]. In such cases, the individual studies are usually small and underpowered and thus, unable to provide a definite answer even in the case where a true association exists. Thus, meta-analysis can effectively combine data from several studies increasing the statistical power (lower type II error rate). An alternative, would be the design of large genetic-association studies possessing the available statistical power to detect a probable association [64]. The particular meta-analysis combined data for more than 13,500 individuals from 30 studies, which is considered a rather large population sample [64] and it was sufficient in order to provide statistically significant results. These results suggest a rather moderate risk associated with the E2 allele and thus, the meta-analysis presented here was able to detect it even if the confidence interval's lower bound is approaching unity in all analyses. Some recently published genome-wide association studies (GWAS) [65–67] as well as a meta-analysis [68] have been performed on sample sizes comparable to the one presented here, but they did not identify ApoE as a major risk allele for T2DM. This however, is something expected since in the GWAS setting the adjustment for multiple testing results in selecting only highly significant markers ($p < 10^{-7}$). On the contrary, for a meta-analysis such as the one presented here which tests only a single marker, a p-value < 0.05 is acceptable.

It is however widely known that bias may be introduced in a meta-analysis pointing to an association that does not exist (type I error). It should be mentioned at this point that every effort has been performed to conduct appropriately the meta-analysis and avoid any possible source of such bias. Quality scoring has not been performed since it is considered subjective [32], non-English articles were identified, retrieved and included in the analysis in order to avoid the local literature bias [31], deviations from the Hardy–Weinberg equilibrium were properly assessed [46] and every appropriate test for detecting publication bias or other small study-related bias were performed [40,69]. Finally, the problem of the early extreme estimates appearing in the meta-analysis of genetic-association studies (the "Proteus phenomenon" or "winner's curse") [44], that correlates with the replication validity of studies in genetic epidemiology [70] was evaluated. The last two forms of bias could severely bias the results of a meta-analysis resulting in a false association; however, no such evidence was observed in this work, strengthening further the validity of the results presented here. Nevertheless, no indication of bias of any kind was identified in this meta-analysis and more importantly, we found no evidence of between-studies heterogeneity.

The exact biological mechanism that underlies this weak but significant association is something that should be investigated. As we already discussed in the introduction, several large meta-analyses have documented that E4 is associated with increased risk for CHD [20,21] and with ICD [22,23]. A previous meta-analysis has shown that subjects carrying the E2 and E4 alleles had lower and higher plasma total cholesterol levels compared to subjects carrying the E3/3 genotype, respectively. Triglycerides concentrations were significantly higher in E2/2, E2/3, E3/4 and E4/2 than in E3/3 subjects. Concurrently, HDL cholesterol was significantly lower in the E3/4 than in the E3/3 individuals [71]. In a recent large meta-analysis, individuals carrying the E2/2 genotype had about 31% lower mean LDL than those with the E4/4 genotype [23].

The results of the present meta-analysis reveal a pattern for T2DM risk that closely resembles the one found concerning triglycerides concentrations (compare Fig. 1 of this article with Fig. 2 in [23] as well as with Fig. 2 in [71]). Insulin resistance is strongly associated with metabolic dyslipidemia and the correlations of lipid profiles with diabetic phenotypes is important, since T2DM patients have an atherogenic lipid profile, which greatly increases their risk of CHD compared to people without diabetes. The largest disparity in lipid levels among people with and without diabetes occurs for HDL and triglycerides: triglycerides tend to be markedly higher and HDL moderately lower in patients with diabetes, in contrast to the negligible difference observed in LDL and TC [28,29]. These results were largely confirmed by our meta-analysis (in addition to these, the mean difference in TC was found to be also significant different from zero). Additional evidence for this correlation came from the "San Antonio Heart Study", in which the cardiovascular risk was determined in subjects who did not have diabetes [72]. Those who developed diabetes during an 8 year follow-up already had higher mean fasting insulin at baseline. Although the differences in fasting glucose were comparatively small between the two groups, they were accompanied by relatively large differences in triglycerides and HDL levels [72]. Moreover, very interesting results on ApoE knock-out and knock-in mice showed that ApoE has also an important role in peripheral energy metabolism and consequently in metabolic syndrome and diabetes [73]. Absence of ApoE reduces body weight and some of their obesity-associated metabolic complications including impaired glucose tolerance and insulin resistance [74,75]. Similar to humans, mice expressing human E3 gain more body weight and adipose tissue mass compared to mice with E4 when following a Western type diet [76]. However, despite being leaner E4 mice begin to show impairment of glucose tolerance earlier than E3 mice, mainly because adipocytes expressing E4 fail to buffer postprandial lipids and glucose completely [76].

The meta-regression approach that we undertook provided some weak evidence that TC, LDL and HDL levels mediate the risk associated with the ApoE variants, even though the results did not reach statistical significance (Fig. 6). However, the results of this analysis are based on only a subset of the studies (12–17 out of the 30 studies) and it is likely that the estimates are attenuated. If complete data were available, perhaps the statistical significance would be reached. Furthermore, meta-regression is prone to ecological confounding [77] and there is an increased probability of an inflation in the type I error rate when several covariates are used, especially when dealing with a small number of studies as is the case here [34,78]. These results may indicate that the genetic effect is larger in studies conducted with control subjects that had lower levels of TC and LDL and higher levels of HDL. After adjusting for control subjects' lipid levels, the association of E2 allele with diabetes was no longer significant, suggesting that it may be mediated by its effect on lipid levels (TC, LDL and HDL). A similar observation has been reported for the associations of ENPP1 and PPARG with diabetes, which seem to mediate their effects through increasing Body Mass Index (BMI) [79,80].

These data are consistent with the view outlined above that correlates Insulin resistance with metabolic dyslipidemia; however, additional and more carefully designed studies are needed in order to establish a more consistent view of these interrelations. For instance, performing large genetic-association studies in T2DM patients and controls, stratified by their lipid profile (HDL, TG, TC and LDL) will minimize the potential confounding by other factors predisposing to components of the metabolic syndrome. Moreover, the Mendelian randomization approach could also be used [81,82] and the causal pathway could be evaluated in more detail. Such an approach could not have been followed here since the included

studies did not report the summary lipid levels stratified for each genotype group. Thus, future studies that take into account the findings of this work need to be performed in order to fully elucidate the biological mechanism of the proposed association.

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