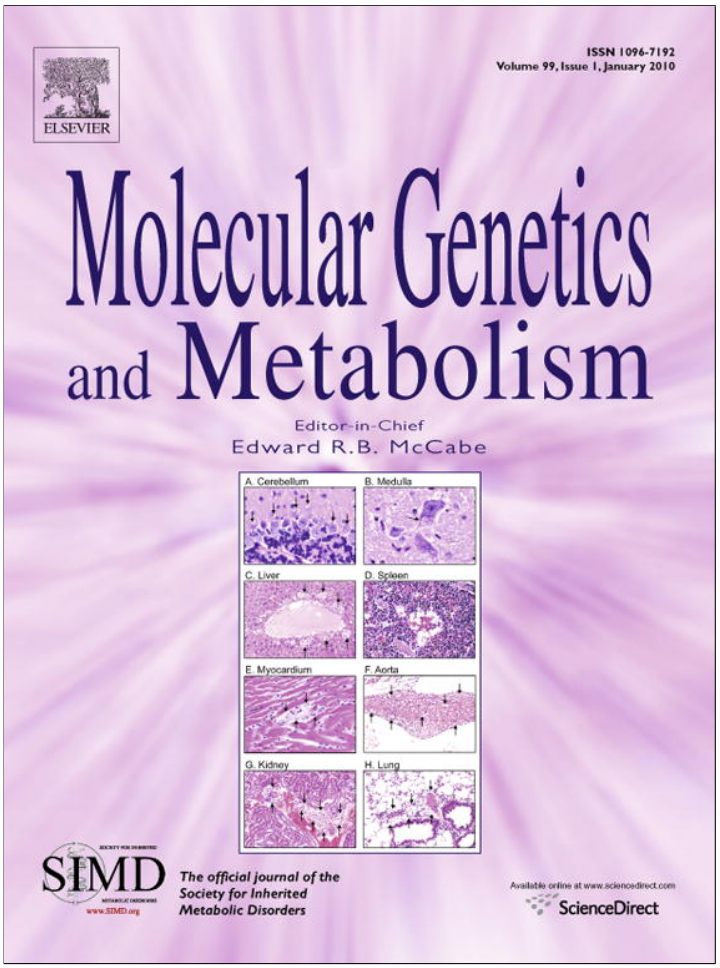

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Influence of hepatocyte nuclear factor 4α (*HNF4α*) gene variants on the risk of type 2 diabetes: A meta-analysis in 49,577 individuals

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ABSTRACT

Background: The nuclear receptor hepatocyte nuclear factor 4α (*HNF4α*) contributes to the regulation of a large fraction of liver and pancreatic islet transcriptomes.
Aim: To evaluate the influence of *HNF4α* polymorphisms across the entire locus on the occurrence of type 2 diabetes (T2D) by means of a meta-analysis.
Methods: We evaluated haplotype block structure of *HNF4α* variants owing to linkage disequilibrium (LD). From 1455 reports, we evaluated 21 observational studies.
Results: Six haplotype blocks of LD were constructed with SNPs with $r^2 > 0.8$; there were also 14 unlinked SNPs. Overall, we included 22,920 cases and 26.657 controls. Among 17 heterogeneous studies (21,881 cases and 24,915 controls), including 3 SNPs of P2 promoter region in block 1, we observed a significant association with T2D in fixed (OR 0.94, 95%CI: 0.905–0.975, $p = 0.001$) and random (OR 0.988, 95%CI: 0.880–0.948, $p = 0.000012$) model. Three homogeneous studies were evaluated in block 2 (2684 cases and 2059 controls), and a significant association with T2D was also observed: OR: 1.121, 95%CI 1.013–1.241, $p = 0.027$. Three additional variants were associated with T2D: two intronic SNPs (rs4810424: OR: 1.080, 95%CI: 1.010–1.154, $p < 0.03$ and rs3212183: OR: 0.843, 95%CI: 0.774–0.918, $p < 0.00009$) and one missense variant (rs1800961: OR: 0.770, 95%CI: 0.595–0.995, $p < 0.05$, 6562 cases and 6723 controls).
Conclusions: In addition to *HNF4α* variants in the promoter region, other SNPs may be involved on the occurrence of T2D.

Introduction

Type 2 diabetes (T2D) is a common, multifactorial disease, with many known and unknown genetic and environmental factors influencing risk.

The discovery of novel T2D genes using whole-genome association studies (GWAS) has provided insight into the genetic architecture of T2D and approximately 20 common variants are now robustly implicated in T2D susceptibility [1].

Additional variants have emerged from meta-analysis of GWAS [2] or combined analysis of biological plausibility with publicly available data from GWAS [3].

Some other candidate genes related to the susceptibility to T2D were explored in the past on the basis of the pathophysiological mechanisms involved in the development of the disease. The nuclear receptor hepatocyte nuclear factor alpha (*HNF4α)* is an example of that. In fact, the protein encoded by *HNF4α* coordinates the expression of several genes required for glucose transport and gluconeogenesis [4,5]. Moreover, *HNF4α* also plays a role in regulating the secretion of insulin by direct activation of the insulin gene promoter [6].

HNF4α, located in chromosome 20: 42,417,855–42,493,444, potentially encodes nine distinct isoforms (*HNF4α1–HNF4α9)* that result from both alternate promoter usage and alternative splicing. Isoforms *HNF4α1–HNF4α6* are coded from the P1 (hepatic) promoter, isoforms *HNF4α7–HNF4α9* are transcribed from the P2 (pancreatic) promoter [7].

Based on both the previous knowledge about mutations of this gene causing the maturity-onset diabetes of the young (MODY) type 1 and results from linkage studies in T2D that showed suggestive linkage peaks in the region of *HNF4α* [8,9], it was speculated that *HNF4α* gene variants may be associated with T2D [10–19]. Most of the gene variants associated with increased T2D risk were identified upstream of the *HNF4α* coding region, in the alternative β-cell promoter P2, which is 46 kb upstream to the P1 promoter of the human gene [20]. However, the evidence for the association of

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polymorphisms in or near P2 region with the risk of T2D was not replicated across all the published studies.

The *HNF4α* gene spans ~76 kb with 10 exons on chromosome 20q13.1–13.2, and in addition to the regulatory region, some other gene variants were also surveyed supporting a role in the T2D predisposition [17,21]. However, a study that evaluated a high density of SNPs spanning the *HNF4α* region failed to replicate any sign beyond the P2 region [22].

Therefore, the significance of the *HNF4α* variants, not only in the P2 promoter haplotype but also along the entire gene locus, in the risk of type 2 diabetes varies in the literature showing that in some populations the *HNF4α* variants do have an effect on disease risk while in other do not. On the hypothesis that any of the *HNF4α* variants could be a putative causal variant or proxy of some others, and to address discrepancies in *HNF4α* genetic association studies, we decided to evaluate the influence of the gene polymorphisms across the entire locus on the occurrence of type 2 diabetes by means of a meta-analysis of all available publications by combining SNPs according to haplotype blocks using the HapMap database (http://www.hapmap.org) whenever possible.

Materials and methods

Data sources and study selection

For the electronic searches, published studies were found through Pubmed at the National Library of Medicine (http://ncbi.nlm.nih.gov/entrez/query) and in Medline databases for the query "(*HNF4α* OR hepatocyte nuclear factor alpha) AND (gene OR variants OR polymorphism OR alleles) AND (type 2 diabetes OR T2D OR non-insulin-dependent diabetes mellitus)". Reference lists in relevant publications were also examined. The literature search was limited to human and was done on studies up to January 2009 and availability of an English-language abstract or paper for review. There were not country restrictions. We first conducted a literature-based systematic review of all relevant studies (irrespective of the SNP typed).

We evaluated 1455 citations identifying 21 studies that met the selection criteria: population-based or hospital-based case-control, cross-sectional studies concerning the relationship between *HNF4α* variants and T2D, in which information about number of subjects in each category, sufficient data to calculate outcomes, and genotyping performed with a validated molecular method could be extracted.

In the case of cohorts, we included variables before any intervention. Data from one further study that fulfilled the eligibility criteria were excluded from the study because data about genotype distribution in cases and controls is disclosed in such a way that precluded further analysis (for instance, calculation of multipoint identities by descent) [16].

An evaluation of study quality of the reviewed articles using the median of the impact factor of journals, in which they had been published, was included [23].

Data collection

All odd ratios (OR) were calculated against healthy control subjects. For each study, information was collected concerning the following characteristics of the subjects: demographic information (age, sex, and ethnicity) and T2D defined as currently taking medication for diabetes or medical record information conforming to World Health Organization (WHO) criteria (World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group* Geneva World Health Org., 1985 (Tech. Rep. Ser., No. 727) or by the oral glucose tolerance test (OGTT) using criteria of the American Diabetes Association (2-h glucose >11.1 mmol/l or fasting glucose >7 mmol/l), as described in each paper. Control subjects were defined either as those who had normal glucose tolerance by (OGTT) or who were normoglycemic.

Statistical analysis

Summary effects, odds ratio (OR) and corresponding 95%CI were estimated by both fixed and random effects meta-analysis using the Mantel–Haenszel method. Heterogeneity was evaluated with *Q* statistic and the I2 statistic, a transformation of *Q* that estimates the percentage of the variation in effect sizes caused by heterogeneity.

Regarding heterogeneity, we identified study characteristics that stratify the studies into subsets with homogeneous effects. We considered possible sources of heterogeneity and stratified the studies by ethnicity, age and gender and repeated the analysis separately for each group. It is worth to mention that because of the conflictive evidence among studies concerning Scandinavian vs Caucasian non-Scandinavian we decided to group these studies in two separated categories if possible. If heterogeneity continued, we ranked the studies according to their individual χ^2, removed the studies with the higher χ^2, and repeated the process until homogeneity was achieved. If the association became homogeneous after stratification or after removing the outliers studies, we recalculated the overall effect and 95%CI, and no further action was taken. Sensitivity of the findings was examined by recalculating the pooled association sizes and joint values of *p* in homogeneous subgroups as well as after excluding one by one study at a time.

All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA). To check for publication bias, we used a visual inspection of funnel plots, the Begg and Mazumdar's rank correlation test [24] and the Egger's regression intercept method [25], but we only show results from the later, as it is the most powerful approach for detecting publication bias.

A *p* value lower than 0.05 was considered statistically significant.

According to the procedure described by Han et al. [26], an *a priori* global estimation of the power for detecting association with the list of analyzed SNPs as possible causal variants was performed assuming an OR of 1.3 for a prevalence of the disease of 10% and on average 3000 cases and 3000 controls, numbers well below the ones including in the meta-analysis. This method gave us an estimated global power greater than 90%.

HNF4α linkage disequilibrium (LD) and haplotype block structure

We evaluated the patterns of LD and haplotype block structure of the *HNF4α* gene variants included in all the published studies evaluated in this meta-analysis. Haplotype block structure was inferred by Haploview available at http://www.broad.mit.edu/mpg/haploview/; in this program, the extent of LD was measured in terms of D', and r^2. To be conservative we constructed blocks of LD with SNPs with $r^2 > 0.8$. SNPs in these blocks are interchangeable and then they can be combined in the meta-analysis irrespective of which one has been genotyped in a particular study [15].

The LD plot, shown in Fig. 1, illustrates six haplotype blocks (B1–B6) in Caucasian population according data from the HapMap project (www.hapmap.org). As available in the HapMap, similar analysis was performed for Japanese subjects (data not shown).

Besides the six LD blocks, we included single SNP that are not in high LD with neighboring variants. Then, we evaluated all the SNPs included in more than one study either belonging to any of the above-mentioned LD blocks or being singleton blocks.

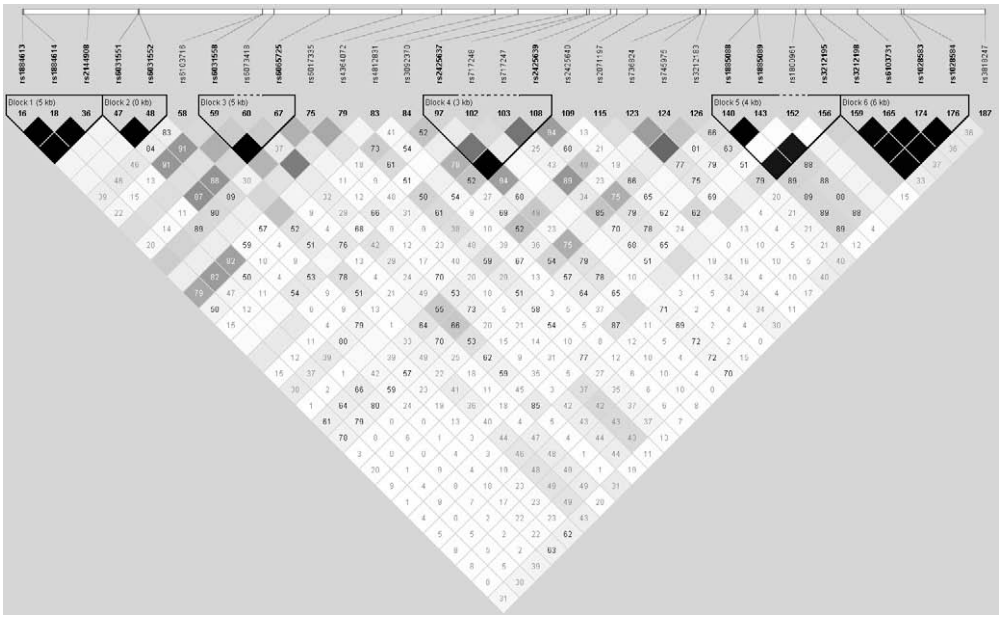


Fig. 1. Basic LD plot across the *HNF4α* locus including all SNPs evaluated in this meta-analysis. LD plot across the *HNF4α* locus region represented by the SNPs included in the study according to the HapMap data for the Caucasian population. The horizontal white line depicts the 77 kb DNA segment of chromosome 20q12–q13.1 analyzed. The location all the SNPs included in analysis is indicated by hatch marks. A linkage disequilibrium plot is depicted in the bottom part of the figure: each diamond represents the magnitude of LD for a single pair of markers, with colors indicating strong LD (black, $r^2 = 1.0$) and no LD (white, $r^2 = 0$) as the extremes (different gray tones indicate intermediate LD). Numbers inside the diamonds stand for D' values 100×.

Results

Meta-analysis of all studies according to haplotype block structure

A complete list of the participating studies is shown in Table 1. LD and haplotype block structure of the *HNF4α* gene variants evaluated in this study are shown in Table 2.

Haplotype block 1

Haplotype 1, composed by 3 SNPs, was represented by 17 heterogeneous studies that encompassed 21,881 cases and 24,915 controls (Table 2, B1) [10–13,15,17–19,21,22,27–33]. Variants in this LD block were significantly associated with T2D either in the fixed (OR 0.94, 95%CI: 0.905–0.975, $p = 0.001$) or in the random model (OR 0.988, 95%CI: 0.880–0.948, $p = 0.000012$), Fig. 2. The heterogeneity was highly significant ($p < 0.0001$) but it was removed by grouping studies by ethnicity (Table 2). Thus, we observed that the association was no significant in Caucasian (19,591 subjects), Japanese (3336 subjects), and Pima Indians (1000 subjects) when studies were stratified by ethnicity. As heterogeneity was still present among the studies that included Caucasian, we removed it by subtracting one study [32] and a sub-sample of another one [19] without significant effect on the effect estimation, which remained not significant and in the opposite direction (data not shown).

Table 1
Characteristics of the included studies.

First author (year)	Reference	Ancestry	Design	Number of cases/controls
Bagwell A.M., 2005	[27]	Caucasian	Hospital based	300/310
Barroso I., 2003	[39]	Caucasian	Population based	1476/1518
Barroso I., 2008	[22]	Mixed: Caucasian and Ashkenazi Jewish	Mixed: population based and family based	1589/1913
Bonnycastle L.L., 2006	[10]	Scandinavian	Mixed: population based and family based	1170/985
Damcott C.M., 2004	[11]	Amish population	Family based	137/342
Hansen S.K., 2005	[12]	Scandinavian	Population based	1429/4727
Hara K., 2006	[13]	Japanese	Hospital based	192/192
Johansson S., 2007	[15]	Scandinavian	Population based	1629/1859
Love-Gregory L.D., 2004	[17]	Ashkenazi Jewish	Family based	275/342
Muller Y.L., 2005	[28]	Pima Indians	Family based	558/450
Qi L., 2007	[29]	Caucasian	Population based	704/1103
Silander K., 2004	[21]	Scandinavian	Mixed: population based and family based	793/409
Takeuchi F., 2007	[30]	Japanese	Hospital based	658/476
Tanahashi T., 2006	[31]	Japanese	Hospital based	925/893
Vaxillaire M., 2005	[32]	Caucasian	Hospital based	734/677
Wanic K., 2006	[33]	Caucasian	Hospital based	461/366
Weedon M.N., 2004	[18]	Caucasian	Population based	2004/1635
Winckler W., 2007	[19]	Scandinavian and Caucasian	Hospital based	3956/3927
WTCCC, 2007	[34]	Caucasian	Mixed: hospital based and population based	1917/2936
Yokoi N., 2006	[40]	Japanese	Hospital based	1590/1244
Zhu Q., 2003	[35]	Japanese	Hospital based	423/353
Total	–	–	–	22,920/26,657

WTCCC, The Wellcome Trust Case Control Consortium.

Table 2
Description of the linkage disequilibrium blocks across the *HNF4α* locus (B1–B6) created by SNPs with $r^2 > 0.8$ among them, showing the chromosomal position for each SNP, the risk and reference alleles, the different ethnic populations and counts for cases and control subjects evaluated in this meta-analysis. Heterogeneity (heterogeneity *p* value) evaluated by *Q* statistic and the I2 statistic, and publication bias, evaluated by Egger's regression intercept method (Egger's *p* value) are also shown.

LD blocks	SNPs	Chr position	Risk/reference alleles	Ethnicity	Cases/controls	Heterogeneity *p* value	Egger's *p* value
B1				Amish	137/342	1.0	0.83
				Ashkenazi Jews	800/800	0.36	
				Caucasian	9714/9877	0.02	
	rs1884613	42413829	C/G	Japanese	1775/1561	0.44	
	rs1884614	42413933	C/T	Pima Indians	554/446	1.0	
	rs2144908	42419131	G/A	Scandinavian	8901/11,889	0.86	
				Total	21,881/24,915	0.0001	
B2				Caucasian	725/673	1.0	0.75
	rs6031551	42423128	T/C	Scandinavian	1959/1386	0.37	
	rs6031552	42423208	C/A	Total	2684/2059	0.21	
B3				Pima Indians	546/443	1.0	0.72
	rs6031558	42433057	G/C	Scandinavian	2801/2841	0.01	
	rs6065725	42438429	G/A	Total	3347/3284	0.006	
B4				Amish	137/342	1.0	0.52
				Ashkenazi Jews	275/342	1.0	
	rs2425637	42457463	T/G	Caucasian	2937/3914	0.96	
	rs2425639	42460924	G/A	Pima Indians	544/440	1.0	
				Scandinavian	3389/6118	0.001	
				Total	7282/11,156	0.006	
B5	rs1885088	42472454		Ashkenazi Jews	275/342	1.0	0.64
	rs1885089	42472677		Caucasian	300/310	1.0	
	rs3212195	42476509	A/G	Caucasian + Scandinavian	3956/3927	1.0	
			T/C	Japanese	925/893	1.0	
			A/G	Pima Indians	548/435	1.0	
				Scandinavian	3380/6059	0.03	
				Total	9384/11,966	0.06	
B6				Amish	137/342	1.0	0.24
				Ashkenazi Jews	275/342	1.0	
	rs3212198	42477776	C/T	Caucasian	2214/3245	0.55	
	rs6103731	42480707	A/G	Caucasian + Scandinavian	3956/3927	1.0	
	rs1028583	42484175	T/G	Japanese	925/893	1.0	
	rs1028584	42484395	A/C	Pima Indians	548/438	1.0	
				Scandinavian	2370/2166	0.55	
				Total	10,425/11,353	0.21	

Chr, chromosomal; AM, Amish; AJ, Ashkenazi Jews; CA, Caucasian; CA–SC, Caucasian + Scandinavian; JA, Japanese; PI, Pima Indians; SC, Scandinavian subjects, respectively.

Haplotype block 2

Haplotype 2 was composed by 2 SNPs. Regarding this block, we evaluated 3 studies [10,21,32], including 2684 cases and 2059 controls (Table 2, B2). A significant association with T2D was observed without evidence of heterogeneity either in the fixed or in the random model (OR: 1.121, 95%CI: 1.013–1.241, $p = 0.027$), Fig. 3.

Haplotype block 3

Haplotype block 3, composed by 2 SNPs (Table 2, B3), was represented by three heterogeneous studies [10,15,28] that included 3347 cases and 3284 controls. No significant association was observed in any of the models, Fig. 4. Most of the heterogeneity seemed owing to the Pima Indian study. However, after subtracting this study heterogeneity still persisted between the remained 2 studies ($p < 0.011$), thus no further action was taken.

Haplotype block 4

Haplotype block 4 composed by 2 SNPs (Table 2, B4) and represented by nine heterogeneous studies (7282 cases and 11,156 controls) [10–12,17,21,27,28,32,34], showed no evidence of association with T2D, Fig. 5. Most of heterogeneity was removed by stratifying the studies by ethnicity but still remained in the 3 Scandinavian studies in which some significant signal was originally reported and it depended on the study that shows an opposite direction of the effect [12].

Haplotype block 5

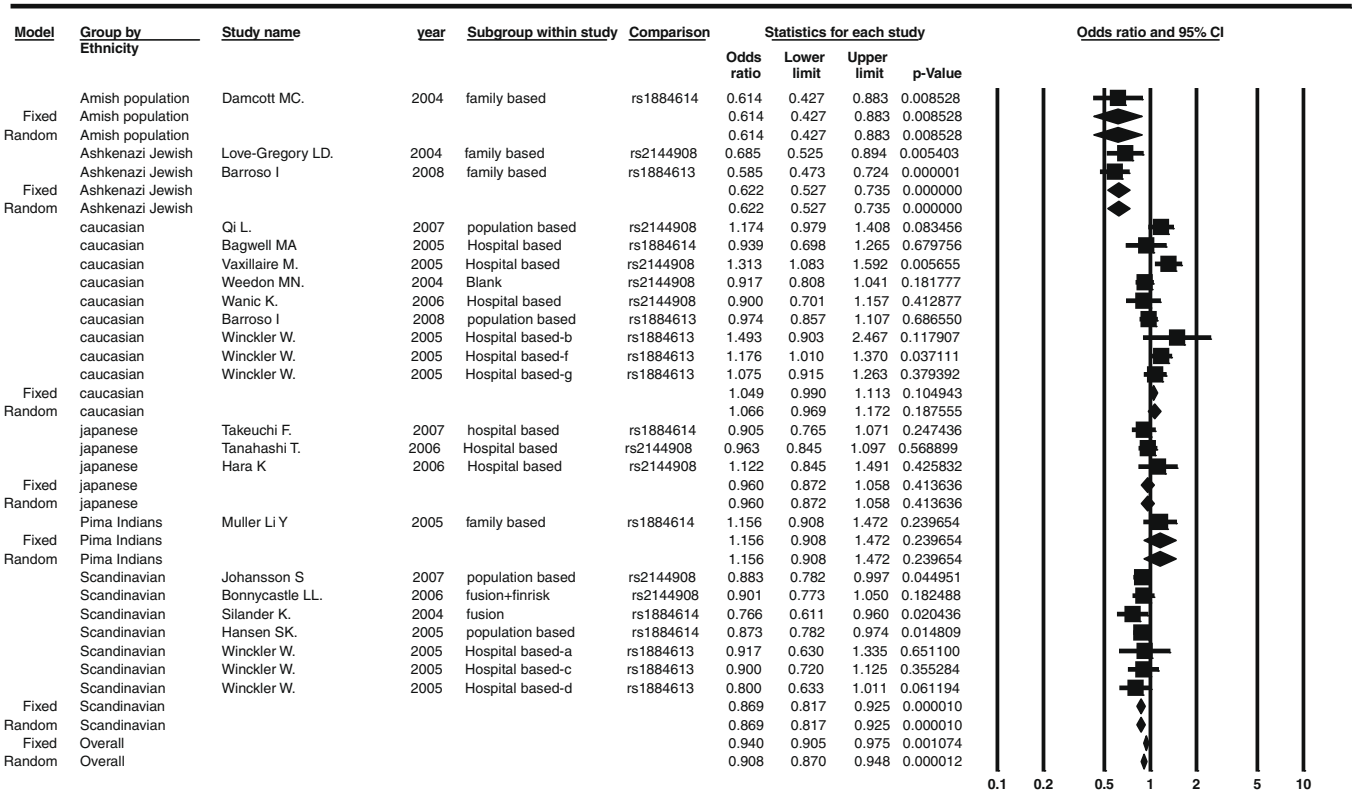
Likewise, haplotype block 5 composed by 3 SNPs (Table 2, B5) and represented by eight homogenous studies [10,12,17,19,21,27,28,31] that included 9384 cases and 11,966 controls showed no significant association with T2D, except a marginal effect in Scandinavian (fixed effect OR 0.913 (95%CI: 0.839–0.995, $p < 0.04$), Fig. 6.

Haplotype block 6

Haplotype 6, composed by 4 SNPs (Table 2, B6) and represented in nine homogeneous studies [11,15,17,19,21,27,28,31,34] that included 10,425 cases and 11,353 controls, was not associated with T2D (Fig. 7). There was no publication bias in none of the studies included in the above-mentioned LD block analysis (Table 2).

Singleton blocks

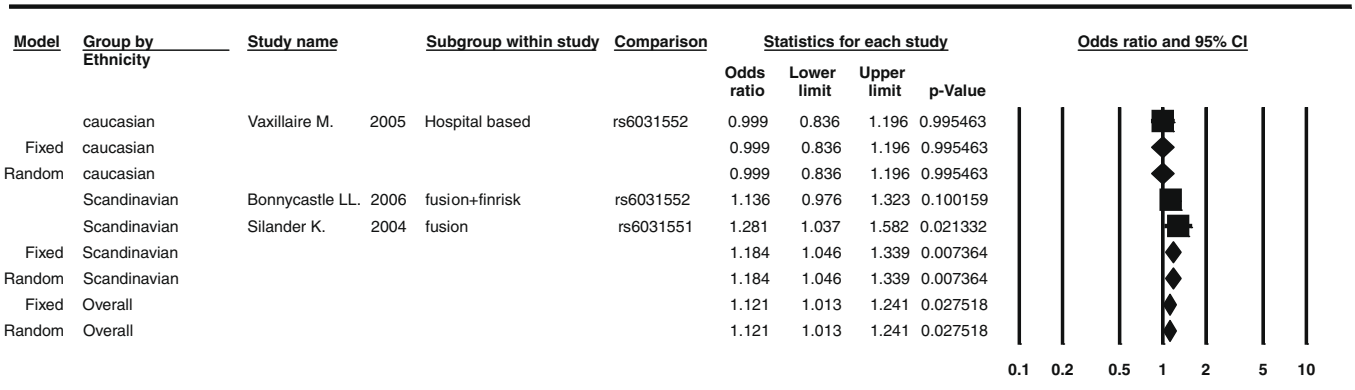
Results concerning the singleton blocks are shown in Table 3. There were three additional variants associated with T2D, two were intronic SNPs (rs4810424 and rs3212183) and one was a missense variant at codon 2 (rs1800961). We observed a low but significant effect for the rs4810424 C allele (fixed model OR: 1.080, 95%CI: 1.010–1.154, $p < 0.03$) without evidence of either heterogeneity (after grouping studies by ethnicity) or publication bias, in 8 studies [13,17,18,21,27,29,31,33] comprising 5632 cases and 5219 controls. In addition, rs3212183-T allele had a protective effect on T2D risk (fixed or random model OR: 0.843, 95%CI: 0.774–0.918, $p < 0.00009$) without evidence of heterogeneity or publication bias



Model	Group by Ethnicity	Study name	year	Subgroup within study	Comparison	Odds ratio	Lower limit	Upper limit	p-Value
	Amish population	Damcott MC.	2004	family based	rs1884614	0.614	0.427	0.883	0.008528
Fixed	Amish population					0.614	0.427	0.883	0.008528
Random	Amish population					0.614	0.427	0.883	0.008528
	Ashkenazi Jewish	Love-Gregory LD.	2004	family based	rs2144908	0.685	0.525	0.894	0.005403
	Ashkenazi Jewish	Barroso I	2008	family based	rs1884613	0.585	0.473	0.724	0.000001
Fixed	Ashkenazi Jewish					0.622	0.527	0.735	0.000000
Random	Ashkenazi Jewish					0.622	0.527	0.735	0.000000
	caucasian	Qi L.	2007	population based	rs2144908	1.174	0.979	1.408	0.083456
	caucasian	Bagwell MA	2005	Hospital based	rs1884614	0.939	0.698	1.265	0.679756
	caucasian	Vaxillaire M.	2005	Hospital based	rs2144908	1.313	1.083	1.592	0.005655
	caucasian	Weedon MN.	2004	Blank	rs2144908	0.917	0.808	1.041	0.181777
	caucasian	Wanic K.	2006	Hospital based	rs2144908	0.900	0.701	1.157	0.412877
	caucasian	Barroso I	2008	population based	rs1884613	0.974	0.857	1.107	0.686550
	caucasian	Winckler W.	2005	Hospital based-b	rs1884613	1.493	0.903	2.467	0.117907
	caucasian	Winckler W.	2005	Hospital based-f	rs1884613	1.176	1.010	1.370	0.037111
	caucasian	Winckler W.	2005	Hospital based-g	rs1884613	1.075	0.915	1.263	0.379392
Fixed	caucasian					1.049	0.990	1.113	0.104943
Random	caucasian					1.066	0.969	1.172	0.187555
	japanese	Takeuchi F.	2007	hospital based	rs1884614	0.905	0.765	1.071	0.247436
	japanese	Tanahashi T.	2006	Hospital based	rs2144908	0.963	0.845	1.097	0.568899
	japanese	Hara K	2006	Hospital based	rs2144908	1.122	0.845	1.491	0.425832
Fixed	japanese					0.960	0.872	1.058	0.413636
Random	japanese					0.960	0.872	1.058	0.413636
	Pima Indians	Muller Li Y	2005	family based	rs1884614	1.156	0.908	1.472	0.239654
Fixed	Pima Indians					1.156	0.908	1.472	0.239654
Random	Pima Indians					1.156	0.908	1.472	0.239654
	Scandinavian	Johansson S	2007	population based	rs2144908	0.883	0.782	0.997	0.044951
	Scandinavian	Bonnycastle LL.	2006	fusion+finrisk	rs2144908	0.901	0.773	1.050	0.182488
	Scandinavian	Silander K.	2004	fusion	rs1884614	0.766	0.611	0.960	0.020436
	Scandinavian	Hansen SK.	2005	population based	rs1884614	0.873	0.782	0.974	0.014809
	Scandinavian	Winckler W.	2005	Hospital based-a	rs1884613	0.917	0.630	1.335	0.651100
	Scandinavian	Winckler W.	2005	Hospital based-c	rs1884613	0.900	0.720	1.125	0.355284
	Scandinavian	Winckler W.	2005	Hospital based-d	rs1884613	0.800	0.633	1.011	0.061194
Fixed	Scandinavian					0.869	0.817	0.925	0.000010
Random	Scandinavian					0.869	0.817	0.925	0.000010
Fixed	Overall					0.940	0.905	0.975	0.001074
Random	Overall					0.908	0.870	0.948	0.000012

Fig. 2. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 1. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-analysis for T2D (comprising 21,327 patients and 24,469 control subjects) and *HNF4α* gene variants of the haplotype block 1. The first row is for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design or different populations. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

in 4 studies [10,11,21,28] including 2608 cases and 2169 controls. Finally, from 5 studies [19,28,29,31,35] including 6562 cases and 6723 controls, we found that the allele C of the rs1800961, which is conserved in several species such as chimp, mouse, rat, and chicken, seemed to confer protection against T2D (random model OR: 0.770, 95%CI: 0.595–0.995, $p < 0.05$). This result should be taken with caution because the studies were heterogeneous, although heterogeneity was removed by grouping studies by eth-



Model	Group by Ethnicity	Study name		Subgroup within study	Comparison	Odds ratio	Lower limit	Upper limit	p-Value
	caucasian	Vaxillaire M.	2005	Hospital based	rs6031552	0.999	0.836	1.196	0.995463
Fixed	caucasian					0.999	0.836	1.196	0.995463
Random	caucasian					0.999	0.836	1.196	0.995463
	Scandinavian	Bonnycastle LL.	2006	fusion+finrisk	rs6031552	1.136	0.976	1.323	0.100159
	Scandinavian	Silander K.	2004	fusion	rs6031551	1.281	1.037	1.582	0.021332
Fixed	Scandinavian					1.184	1.046	1.339	0.007364
Random	Scandinavian					1.184	1.046	1.339	0.007364
Fixed	Overall					1.121	1.013	1.241	0.027518
Random	Overall					1.121	1.013	1.241	0.027518

Fig. 3. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 2. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-analysis for type 2 diabetes (comprising 2684 patients and 2059 control subjects) and *HNF4α* gene variants of the haplotype block 2. The first row is for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design or different populations. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

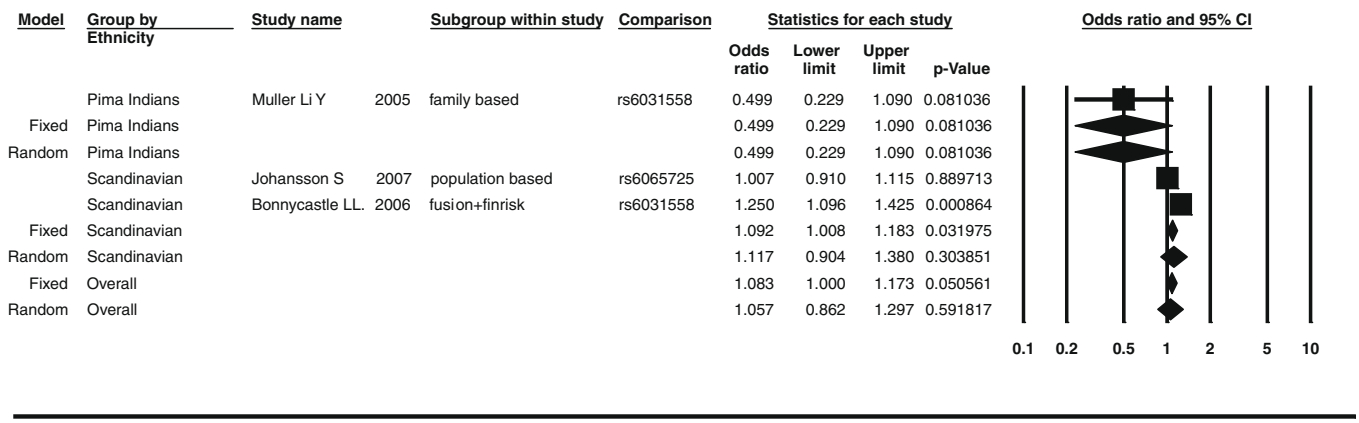


Fig. 4. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 3. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-regression analysis for type 2 diabetes (comprising 3347 patients and 3284 control subjects) and *HNF4α* gene variants of the haplotype block 3. The total number of combined studies is indicated between parentheses, the first row for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design or different populations. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

nicity. There was a moderate publication bias owing to the first report by Zhu et al. [35].

Overall study quality

The median impact factor for all the included studies was 8.107 (range 1.710–28.751).

Discussion

Although *HNF4α* P1/P2 promoter SNPs have been described in previous T2D association studies, and a meta-analysis of P2 regulatory region showed that promoter gene variants are associated with T2D only in Scandinavians subjects [15], a methodical examination of the entire locus has not been yet reported. Because other variants beyond the promoter region may confer an additional risk of T2D in different populations, we performed a systematic review of the literature by means of a meta-analysis on the relationship of *HNF4α* gene variants across the entire locus on the occurrence of T2D. This review evaluated a total of 49,577 subjects, including 22,920 cases and 26,657 control subjects.

According with the previous discussed evidence, we observed that variants in P2 region (SNPs in haplotype block 1) were significantly associated with T2D in 17 heterogeneous studies encompassing 21,327 cases and 24,469 control subjects, without

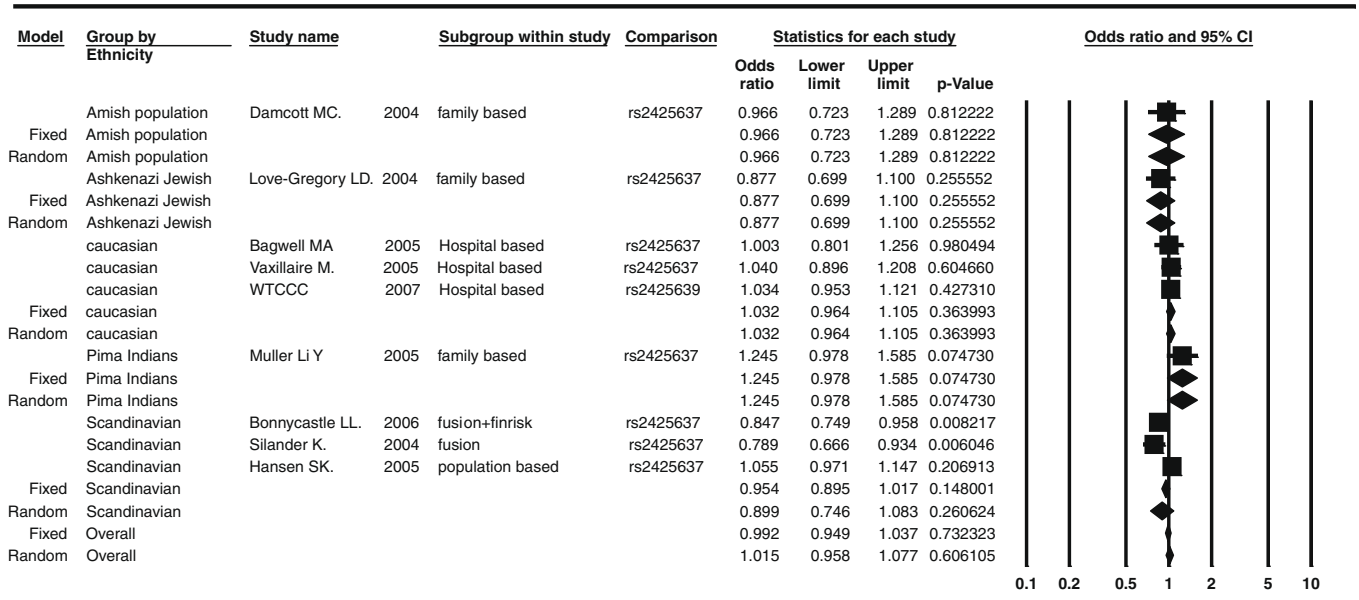


Fig. 5. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 4. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-analysis for type 2 diabetes (comprising 7382 patients and 11,156 control subjects) and *HNF4α* gene variants of the haplotype block 4. The total number of combined studies is indicated between parentheses, the first row for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design or different populations. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

Model	Group by Ethnicity	Study name	year	Subgroup within study	Comparison	Odds ratio	Lower limit	Upper limit	p-Value
	Ashkenazi Jewish	Love-Gregory LD.	2004	family based	rs1885088	1.176	0.896	1.543	0.242429
Fixed	Ashkenazi Jewish					1.176	0.896	1.543	0.242429
Random	Ashkenazi Jewish					1.176	0.896	1.543	0.242429
	caucasian	Bagwell MA	2005	Hospital based	rs1885088	1.069	0.812	1.406	0.636296
Fixed	caucasian					1.069	0.812	1.406	0.636296
Random	caucasian					1.069	0.812	1.406	0.636296
	caucasian+scandinavian	Winckler W.	2005	Hospital based	rs1885088	0.952	0.833	1.087	0.468756
Fixed	caucasian+scandinavian					0.952	0.833	1.087	0.468756
Random	caucasian+scandinavian					0.952	0.833	1.087	0.468756
	japanese	Tanahashi T.	2006	Hospital based	rs1885088	2.079	0.779	5.551	0.144162
Fixed	japanese					2.079	0.779	5.551	0.144162
Random	japanese					2.079	0.779	5.551	0.144162
	Pima Indians	Muller Li Y	2005	family based	rs1885088	0.951	0.779	1.161	0.622334
Fixed	Pima Indians					0.951	0.779	1.161	0.622334
Random	Pima Indians					0.951	0.779	1.161	0.622334
	Scandinavian	Bonnycastle LL.	2006	fusion+finrisk	rs1885088	0.833	0.696	0.996	0.045486
	Scandinavian	Silander K.	2004	fusion	rs1885088	0.700	0.541	0.907	0.006943
	Scandinavian	Hansen SK.	2005	population based	rs1885088	0.984	0.886	1.092	0.761104
Fixed	Scandinavian					0.913	0.839	0.995	0.036894
Random	Scandinavian					0.855	0.709	1.031	0.101430
Fixed	Overall					0.950	0.891	1.012	0.113118
Random	Overall					0.967	0.888	1.053	0.436672

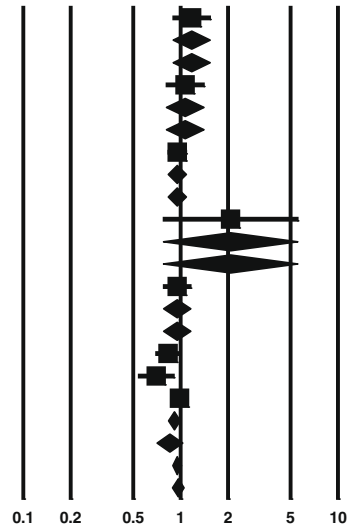


Fig. 6. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 5. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-analysis for type 2 diabetes (comprising 9384 patients and 11,966 control subjects) and *HNF4α* gene variants of the haplotype block 5. The total number of combined studies is indicated between parentheses, the first row for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design of different SNP. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

Model	Group by Ethnicity	Study name	year	Subgroup within study	Comparison	Odds ratio	Lower limit	Upper limit	p-Value
	Amish population	Damcott MC.	2004	family based	rs1028583	1.138	0.855	1.514	0.376873
Fixed	Amish population					1.138	0.855	1.514	0.376873
Random	Amish population					1.138	0.855	1.514	0.376873
	Ashkenazi Jewish	Love-Gregory LD.	2004	family based	rs1028583	1.290	1.001	1.662	0.048895
Fixed	Ashkenazi Jewish					1.290	1.001	1.662	0.048895
Random	Ashkenazi Jewish					1.290	1.001	1.662	0.048895
	caucasian	Bagwell MA	2005	Hospital based	rs3212198	1.025	0.817	1.285	0.831973
	caucasian	WTCCC	2007	Hospital based	rs1028583	0.953	0.877	1.035	0.249933
Fixed	caucasian					0.961	0.889	1.038	0.313358
Random	caucasian					0.961	0.889	1.038	0.313358
	caucasian+scandinavian	Winckler W.	2005	Hospital based	rs1028583	1.087	0.970	1.218	0.152006
Fixed	caucasian+scandinavian					1.087	0.970	1.218	0.152006
Random	caucasian+scandinavian					1.087	0.970	1.218	0.152006
	japanese	Tanahashi T.	2006	Hospital based	rs3212198	0.911	0.794	1.045	0.182863
Fixed	japanese					0.911	0.794	1.045	0.182863
Random	japanese					0.911	0.794	1.045	0.182863
	Pima Indians	Muller Li Y	2005	family based	rs3212198	0.962	0.802	1.154	0.677237
Fixed	Pima Indians					0.962	0.802	1.154	0.677237
Random	Pima Indians					0.962	0.802	1.154	0.677237
	Scandinavian	Johansson S	2007	population based	rs1028583	0.980	0.887	1.083	0.688513
	Scandinavian	Silander K.	2004	fusion	rs1028583	0.921	0.775	1.095	0.352498
Fixed	Scandinavian					0.965	0.885	1.052	0.416680
Random	Scandinavian					0.965	0.885	1.052	0.416680
Fixed	Overall					0.989	0.945	1.034	0.619331
Random	Overall					0.989	0.945	1.034	0.619331

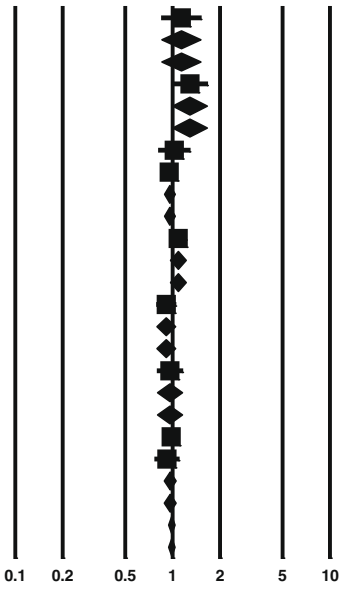


Fig. 7. Meta-analysis plot of *HNF4α* case/control studies included in haplotype block 6. Summary estimates for odds ratios (effect), the corresponding 95%CI limits (lower and upper) and significance (*p* value) were calculated by fixed and random effects MH meta-analysis for type 2 diabetes (comprising 10,425 patients and 11,353 control subjects) and *HNF4α* gene variants of the haplotype block 6. The total number of combined studies is indicated between parentheses, the first row for fixed and the second one for random effects, respectively. The first author of the study is indicated under citation. The year of the publication is also shown. The first author of the study appears more than once in the figure when the data was regarded separately either by study design of different SNP. In the graph, numbers indicate OR in a log scale, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

Table 3
Summary estimates for OR, the corresponding 95%CI limits (lower and upper) and significance (*p* value) were estimated by fixed and random effects analysis for Type 2 diabetes risk in relation to the single variants in *HNF4* evaluated in each study included in the meta-analysis.

NCBI SNP reference[a]	Location in the *HNF4* gene	Chromosome position	Risk allele	References	Cases/ controls	Association test Fixed and random OR (95%CI, *p* value)	Heterogeneity *p* value	Egger's *p* value
rs3818247	Intronic	42490894	G	[10–13,15,17,19,21,27,28,30]	11,012/ 13,977	1.029 (0.983–1.076, $p = 0.21$)	0.016	0.21
rs4810424	Intronic	42408437	C	[13,17,18,21,27,29,31,33]	5632/ 5219	1.080 (1.010–1.154, $p = 0.024$) 1.081 (1.007–1.162, $p = 0.032$)	0.009	0.37
rs2425640	5' near gene	42461451	A	[10,11,17,21,27,28]	3212/ 2835	0.931 (0.859–0.1.008, $p = 0.07$)	0.007	0.03
rs3212183	Intronic	42468552	T	[10,11,21,28]	2608/ 2169	0.843 (0.774–0.918, $p = 0.000087$)	NS	0.80
rs4812831	Intronic	42451674	G	[13,15,28]	2352/ 2471	0.920 (0.817–1.037, $p = 0.17$)	0.017	0.71
rs6017335	Intronic	42444239	A	[15,28]	2170/ 2296	1.058 (0.967–1.158, $p = 0.21$)	NS	NA
rs6103716	Intronic	42433044	C	[10,15]	2756/ 2808	1.095 (1.013–1.184, $p = 0.023$) 1.121 (0.893–1.407, $p = 0.32$)	0.005	NA
rs717247	Intronic	42459198	G	[15,27,40]	3519/ 3413	0.995 (0.925–1.071, $p = 0.90$)	NS	0.91
rs717248	Intronic	42459019	G	[15,27]	1925/ 2158	0.861 (0.671–1.104, $p = 0.23$)	NS	NA
rs2071197	Intronic	42463849	G	[28,31,39]	1923/ 1840	1.051 (0.952–1.160, $p = 0.32$)	0.001	0.62
rs736824	Intronic	42468074	G	[27,31,39]	1735/ 1708	0.994 (0.899–1.101, $p = 0.91$)	NS	0.90
rs745975	Intronic	42468107	A	[27,39,40]	2397/ 2056	1.053 (0.949–1.167, $p = 0.33$) 1.070 (0.959–1.194, $p = 0.22$).	0.045	0.57
rs6073418	Intronic	42434004	T	[13,27,31]	1418/ 1395	0.950 (0.840–1.075, $p = 0.41$)	NS	0.85
rs1800961	Missense Ile [I]/Thr [T] Codon 2	42475778	C	[19,28,29,31,35]	15,020/ 15,010	0.871 (0.718–1.056, $p = 0.160$) 0.770 (0.595–0.995, $p = 0.045$)	0.025	0.03
rs4364072	Intronic	42447380	G	[19,28]	4502/ 4360	0.997 (0.894–1.111, $p = 0.95$)	NS	NA
rs3092370	Intronic	42453517	A	[15,19]	5582/ 5773	0.958 (0.892–1.028, $p = 0.23$)	NS	NA

NS, no significant.
Heterogeneity *p* value was evaluated with Q statistic and the I2 statistic and Egger's regression intercept method was used to check for publication bias (Egger's *p* value). A *p* value lower than 0.05 was considered statistically significant.
[a] Single nucleotide polymorphisms on NCBI reference assembly.

publication bias. The haplotype CCG of block 1 confers an overall protection against T2D of 9% (OR: 0.908). Again, the evidence showed that the association was not observed either in Caucasian (19,591 subjects), Japanese (3336 subjects) or Pima Indians (1000 subjects). By the contrary, the effect was almost restricted to Amish (479 subjects, one study), Scandinavians (20,790 subjects) and Ashkenazi Jewish (1600 subjects). Likewise, variants in P2-haplotype block 2 showed a significant association with T2D only in Scandinavians. Results from the available data included in this meta-analysis showed that no further association with type 2 diabetes was observed along the remaining LD blocks.

As stated by Barroso et al. [22], a remarkable point regarding these results is that ethnicity may act as an important variable in determining association risk with T2D, as the effect is systematically negative in Caucasian-non-Scandinavian subjects. We cannot explain how ethnicity influences the *HNF4*α-related genetic risk of T2D in some but not all populations, besides the well know influence of lifestyle factors such as diet and exercise. However, previous data support the biological basis for the ethnic differences in the risk of T2D [36]. For instance, there is evidence about differences in postprandial glycemia and insulin sensitivity among young adults of different ethnic origins [37].

In addition, despite not particularly related to T2D, Scandinavian subjects show a greater susceptibility to specific diseases, such as Crohn disease or intrahepatic cholestasis of pregnancy, than the prevalence observed in other Caucasian populations. Thus, we cannot rule out the additional weight that environmental factors may have in the etiology of common diseases.

It is worthy to note that these findings argue against reporting genetics analysis from mixed populations even though they were from northern and western European ancestry [19] and calls for the needs of extending the HapMap initiative to include samples representative of people around the whole world.

Interestingly, when we evaluated the effect of singleton blocks located beyond the regulatory region on the occurrence of T2D, we detected 3 positive associations with T2D. Two of them were intronic SNPs (rs4810424 and rs3212183); the association for rs4810424 was also observed in Caucasian (3451 cases and 3383 control subjects) although with a more modest effect (OR: 1.11 for the C allele) than in Scandinavian (OR: 1.31) being in the oppo-

site direction in Ashkenazi Jewish subjects [17]. The T allele of the rs3212183 intronic variant showed a consistent and protective effect across Amish, Pima Indians and Scandinavian populations (OR: 0.84). The third variant significantly associated with T2D in 6562 patients and 6723 control subjects was rs1800961, a nonsynonymous variant (I139T) at codon 2. The C allele of this SNP, initially described as a loss-of-function mutation in hepatocytes [35], is conserved in different species such as chimp, mouse, rat and chicken and seems to confer protection against T2D in this study. This effect was mostly observed in Japanese and Pima Indians subjects. Nevertheless, this association was observed only in the random model among six heterogeneous studies. In this regard, a note of caution should be added because heterogeneity may potentially restrict the interpretation of the pooled risk estimates. Heterogeneity in a meta-analysis is mostly produced by differences in study design and background characteristics of the subjects and the extent of heterogeneity might influence the conclusions. However, a random effect model, where heterogeneity is no longer an issue, provided a significant result.

Some weaknesses of our study should be discussed. First, we used the HapMap data to define the haplotype structure of the entire 77 kb genomic sequence. While no other available information apart from Caucasian and Japanese populations that were both used in this paper, it may be questionable the rationale of using the HapMap data in so genetically heterogeneous populations. However, the HapMap is now the only instrument available for the haplotype construction. Second, as we evaluated 22-test (16 singleton plus 6 blocks) adjustment for multiple testing should be investigated. Nevertheless, even using the more conservative approach such as Bonferroni correction and then an empiric p value of 0.0023 (0.05/22), the main conclusions of the study still hold.

Finally, it is worth mentioning that *HNF4α*, which acts as a master switch regulating the expression of hundreds of other genes, was previously associated with either T2D or lipid levels, glucose parameters and BMI in large cohorts of individuals in both case-control and family studies. Hence, it is not surprising that *HNF4α* plays an important role in the pathophysiology of T2D. Moreover, this gene is a master regulator of hepatocyte and islet transcription network of many of the central rate-limiting steps in gluconeogenesis and associated pathways [38], what suggest a mechanistic explanation for the findings in our meta-analysis.

In conclusion, we have confirmed in a large sample that the effect on T2D risk of the haplotype block surrounding the alternate P2 promoter is ethnicity dependent. In addition, we found that some other variants beyond this region may be associated with the risk of T2D, which deserves further investigation.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

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