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Association of the calpain-10 gene with type 2 diabetes in Europeans: Results of pooled and meta-analyses

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Abstract

We conducted pooled and meta-analyses of the association of the calpain-10 gene (*CAPN10*) polymorphisms SNP-43, Indel-19 and SNP-63 individually and as haplotypes with type 2 diabetes (T2D) in 3237 patients and 2935 controls of European ancestry. In the pooled analyses, the common SNP-43*G allele was associated with modest but statistically significant increased risk of T2D (odds ratio (OR) = 1.11 (95% confidence interval (CI), 1.02–1.20), $P = 0.01$). Two haplotype combinations were associated with increased risk of T2D (1-2-1/1-2-1, OR = 1.20 (1.03–1.41), $P = 0.02$; and 1-1-2/1-2-1, OR = 1.26 (1.01–1.59), $P = 0.04$) and one with decreased risk (1-1-1/2-2-1, OR = 0.86 (0.75–0.99), $P = 0.03$). The meta-analysis also showed a significant effect of the 1-2-1/1-2-1 haplogenotype on risk (OR = 1.25 (1.05–1.50), $P = 0.01$). However, there was evidence for heterogeneity with respect to this effect ($P = 0.06$). The heterogeneity appeared to be due to data sets in which the cases were selected from samples used in linkage studies of T2D. Using only the population-based case-control samples removed the heterogeneity ($P = 0.89$) and strengthened the evidence for association with T2D in both the pooled (SNP-43*G, OR = 1.19 (1.07–1.32), $P = 0.001$; 1-2-1/1-2-1 haplogenotype, OR = 1.46 (1.19–1.78), $P = 0.0003$; 1-1-2/1-2-1 haplogenotype, OR = 1.52 (1.12–2.06), $P = 0.007$; and 1-1-1/2-2-1 haplogenotype, OR = 0.83 (0.70–0.99), $P = 0.03$) and the meta-analysis (SNP-43*G, OR = 1.18 (1.05–1.32), $P = 0.005$; 1-2-1/1-2-1 haplogenotype, OR = 1.68 (1.33–2.11), $P = 0.00001$). The pooled and meta-analyses as well as the linkage disequilibrium and haplotype diversity studies suggest a role for genetic variation in *CAPN10* affecting risk of T2D in Europeans.

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Introduction

Our genes determine, at least in part, our risk of developing type 2 diabetes (T2D) [1]. It is commonly believed that the identification of the genes and associated variants that modify risk (increase or decrease) will lead to rational approaches for preventing and treating diabetes that take into account the individual's genetic risk profile. We have been studying the genetics of T2D in the Mexican American population of Starr County, Texas [2–4]. A genome-wide search for T2D susceptibility loci mapped a gene(s) with a major effect on risk, designated *NIDDM1*, to the distal long arm of chromosome 2 (band 2q37.3) [2]. This locus was estimated to have a sibling recurrence risk (λ_s) of 1.37 (1-lod confidence interval for λ_s, 1.13–1.74) for an additive and 1.36 (1.14–1.67) for a non-additive model. *NIDDM1* could account for 21–30% of the familial clustering of T2D in the population studied, depending on the model (additive or multiplicative) for the interaction between *NIDDM1* and other susceptibility loci. We positionally cloned a gene in the *NIDDM1* region that showed both linkage and association with T2D [4]. This gene, calpain-10 (*CAPN10*),[1] encoded a nonlysosomal cysteine protease that was expressed in all tissues examined, including tissues such as pancreatic islets, liver, skeletal muscle and fat that play an important role in the regulation of glucose homeostasis [4,5].

We showed in this first report that risk could be most readily defined in terms of specific haplotypes of three polymorphisms: SNP-43, Indel-19 and SNP-63. The association of these SNPs and their haplotypes with T2D has not been consistently replicated [6–11]. The inconsistency has been a troubling feature of genetic studies of *CAPN10* and T2D and has raised doubts that genetic variation in *CAPN10* is indeed associated with T2D. However, two recent meta- and pooled-analyses in large groups of cases and controls (>3500 each) have shown association of two SNPs in calpain-10 (SNP-43 and -44) with T2D [12,13]. Thus, the data look more convincing as larger sample sizes have been assembled, although as noted by McCarthy et al. [14] "the combined evidence is not, as yet, incontrovertible." This is especially true of the genetic model in which Mexican Americans with the haplogenotype (SNP-43, Indel-19, SNP-63) designated 1-1-2/1-2-1 had the highest risk and those homozygous for either the 1-1-2 or 1-2-1 haplotype were not at increased risk. However, Mexican Americans are an admixed population (the ancestral contributions to the contemporary gene pool of the Mexican American population of Starr County population are 61% Spanish, 31% Native American and 8% African [15]) and this may be a confounding factor in both the original and in subsequent studies. Thus, small samples sizes and the unique nature of the Mexican American population may be responsible for the failure to obtain consistent replication. In order to gain a better understanding of the contribution of genetic variation in *CAPN10* to risk of T2D, we have begun to carry out large association studies in diverse populations with the view that different variants and haplotypes may affect risk in different populations. Here, we report the association testing of SNP-43, Indel-19 and SNP-63, individually and by haplotype with T2D in individuals of European ancestry supplemented by an examination of linkage disequilibrium and haplotype diversity of *CAPN10* in this population.

Materials and methods

Subjects

We considered all published case-control studies that examined the association of the *CAPN10* polymorphisms SNP-43, Indel-19 (or SNP-56 which is in perfect linkage disequilibrium (LD) with Indel-19) and SNP-63 with T2D in populations of European (Caucasian) ancestry [4,6–11,16]. We did not include SNP-44 which has been independently associated with T2D [9] as it was not typed in four of the datasets representing 53.8% of cases and 33.3% of controls: Denmark, Finland (FUSION), Poland and France (Table 1). We excluded the study of Elbein et al. [8] which was a family-based study of approximately 700 members of 63 families in which *CAPN10* variants were tested for linkage and association (transmission disequilibrium test) with T2D rather than a case-control study. We also excluded the study of Berger et al. [16] because the cases were ascertained for both T2D and end-stage kidney disease. We contacted the primary investigator of each study and obtained the raw genotype data so that we could carry out both pooled and meta-analyses. We also genotyped SNP-43, Indel-19 and SNP-63 in an additional 2,514 subjects from five case-control studies (one Czech, one French and three German). There was overlap between the Finnish and German subjects reported in Horikawa et al. [4] and the Botnia-1 and Germany-1 cohorts, respectively, and we therefore used only the larger data sets (i.e., Botnia-1 and Germany-1) which removed all overlap. The clinical characteristics of the study populations are summarized in Table 1. The genotype and haplotype distributions for all the study populations are provided in Tables 1 and 2 in the online supplementary material.

Statistical analyses

We tested each polymorphism (in each data set and in the pooled sample) for deviation from HWE. We then tested for differences in allele, genotype, haplotype and haplogenotype frequencies between groups (within study and pooled) using a chi-squared test. Previous studies have shown that there are only four common SNP-43–Indel-19–SNP-63 haplotypes observed in Mexican Americans, Japanese and Europeans rather than the eight expected permitting unambiguous haplotype assignment. The four observed haplotypes are described as 1-1-1, 1-1-2, 1-2-1 and 2-2-1 [4]. We

[1] *Abbreviations used: CAPN10*, symbol for the human calpain-10 gene; Indel, insertion/deletion polymorphism; CI, confidence interval; Ctrl, controls; Het, heterogeneity; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; MAF, minor allele frequency; NGT, normal glucose tolerance; OR, odds ratio; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

removed subjects with missing genotypes (86 cases and 49 controls) or the rare haplotypes 1-2-2, 2-2-2, 2-1-1 and 2-1-2 (an indication of possible genotyping error) (19 cases and 14 controls) from the haplotype-based analyses. We used the odds ratio as a measure of risk. In the meta-analysis, summary odds ratios and 95% confidence intervals were estimated under both fixed effects (Mantel–Haenszel) and random effects models (DerSimonian–Laird). We tested for heterogeneity across comparisons using the chi-squared-based Q statistic and heterogeneity was considered significant for $P < 0.10$. Publication bias was assessed using Begg's adjusted rank correlation test and Egger's regression asymmetry test using the STATA software. All *P*-values are two-sided.

Linkage disequilibrium and haplotype blocks

To place the pooled and meta-analyses of SNP-43, Indel-19 and SNP-63 in the context of the overall pattern of linkage disequilibrium (LD) in the *CAPN10* region, we typed 30 polymorphisms (SNPs and Indels) in this region in a group of 72 unrelated healthy individuals of European ancestry residing in San Francisco, CA using Taqman-based assays (for SNPs) and agarose gel electrophoresis (for Indels). The genotype distributions of all polymorphisms were in Hardy–Weinberg equilibrium (HWE), $P > 0.05$. We calculated pairwise measures of LD, visualized the patterns of LD using Haploview [http://www.broad.mit.edu/mpg/haploview/index.php] [17], and defined haplotype blocks using the confidence interval algorithm [18] and the four-gamete rule [19]. The confidence interval defines haplotype blocks as follows. For each marker pair, if the one-sided 95% confidence interval for D′ had an upper bound >0.98 and lower bound >0.7, the marker pairs were deemed to be in strong LD. If the upper bound was <0.9, the marker pair was considered to have undergone historic recombination. All other marker pairs were labeled inconclusive. A block was created if 95% of informative (i.e., non-inconclusive) comparisons of marker pairs with minor allele frequencies >0.05 were considered to be in strong LD. The four-gamete rule defines haplotype blocks by the absence of all four two-marker haplotypes for each marker pair occurring at frequency >0.01. We used Tagger [20] in Haploview [17] to determine the proportion of markers in the block that could be captured (having r^2 greater than the particular threshold set) by forcing SNP-44, SNP-43, Indel-19, and SNP-63 as tag SNPs. LD patterns were also compared with those observed in the Europeans in HapMap [www.hapmap.org] [21].

We determined frequencies of haplotypes spanning the 15 marker block surrounding *CAPN10* in the 63 individuals missing less than 20% of their genotype calls using PHASE 2.0.2 [http://www.stat.washington.edu/stephens/software.html] [22,23] with the default number of iterations, burn-in, and thinning interval. Consistency of phase assignment was assessed through five runs from different random seeds.

Results

Association of SNPs in CAPN10 with type 2 diabetes

We performed both pooled and meta-analyses of 12 data sets including 3237 cases and 2935 controls (Table 1). We carried out both types of analysis because pooling increases the power to detect and quantify an effect and the meta-analysis provides a control for population differences that could lead to spurious association if there are differences in allele frequency among groups. The power of detecting an effect in a sample of this size in the overall pooled analyses for SNP-43, Indel-19 and SNP-63 was 0.71, 0.06 and 0.69, respectively; and 0.22, 0.61, 0.68 and 0.65 for the 1-1-1, 1-2-1, 1-1-2 and 2-2-1 haplotypes described below [24]. Power

Table 1
Clinical characteristics of European study populations

Reference	Population	Selection/characteristics of cases (mean age-at-diagnosis) and controls (mean age)		Eligible subjects	
		T2D	Ctrl	T2D	Ctrl
Horikawa et al. [4]	Germany (Germany-1)	T2D, Probands of sibs, (42.7 ± 12.7 years)	Random, (50.8 ± 11.9 years)	308	88
	Finland (Botnia-1)	T2D, age-at-diagnosis ⩾40 years, (54.6 ± 8.6 years)	NGT per WHO criteria, (60.6 ± 9.1 years)	192	192
Evans et al. [6]	UK (UK-1)	T2D, Probands of trios, (40.2 ± 7.1 years)	Population, birth cohort (0 ± 0.0 years)	153	411
	UK (UK-2)	T2D, Probands of sibs, age-at-diagnosis ⩾40 years, (55.3 ± 8.5 years)	—	222	—
Orho-Melander et al. [11]	Finland (Botnia-2)	T2D, (51.4 ± 11.8 years)	NGT per WHO criteria, (60 ± 10 years)	203	106
Rasmussen et al. [7]	Denmark	T2D, (55.3 ± 10.6 years)	NGT per WHO criteria, (52.0 years)	409	200
Fingerlin et al. [9]	Finland (FUSION)	T2D, Probands of sibs from the first and second sets, (50.7 ± 8.4 years)	NGT per WHO criteria, Unaffected spouses, (61.7 ± 7.7 years)	784	186
	Finland (FUSION)		NGT per WHO criteria, Elderly controls, (70.0 ± 0.3 years)	—	223
Malecki et al. [10]	Poland	T2D, (46.3 ± 10.6 years)	Non-diabetic, (56.5 ± 14.3 years)	229	148
Unpublished	Czech Republic	T2D, (50.1 ± 8.7 years)	NGT per WHO criteria (18.1 ± 2.3 years)	259	143
Unpublished	France	T2D, (45.8 ± 12.5 years)	Normoglycemic, (62 years)	175	220
Unpublished	France	T2D, Probands of sibs, (49.8 ± 9.9 years)	—	143	—
Unpublished	Germany (Germany-2)	T2D, (63.4 ± 11.9 years)	NGT per WHO criteria, (42.2 ± 17.2 years)	133	631
Unpublished	Germany (Germany-3)	T2D, (62.1 ± 6.5 years)	NGT per WHO criteria, (58.7 ± 8.4 years)	27	387

SNP-56 was typed instead of Indel-19 in the FUSION samples. Ctrl, controls; T2D, type 2 diabetes; NGT, normal glucose tolerance; WHO, World Health Organization. For the meta-analysis, we combined the FUSION ctrl groups and the French case (in the overall comparison) groups.

Table 2
CAPN10 polymorphisms and risk of type 2 diabetes in Europeans stratified based on ascertainment of cases (population-based vs. linkage-based)

Marker	Genotype/allele	Overall									Population-based cases								
		n or frequency		Pooled		Meta-analysis					*n* or frequency		Pooled		Meta-analysis				
						Fixed effects		Random effects		Het					Fixed effects		Random effects		Het
		Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*	Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*
SNP-43	G/G	1496	1657								994	886							
	G/A	1178	1172								844	608							
	A/A	247	226		0.05*						186	126		0.003*					
	G	0.714	0.734	1.11 (1.02–1.20)	0.01	1.08 (0.99–1.18)	0.09	1.08 (0.99–1.18)	0.09	0.43	0.700	0.735	1.19 (1.07–1.32)	0.001	1.18 (1.05–1.32)	0.005	1.18 (1.05–1.32)	0.005	0.61
	A	0.286	0.266	0.90 (0.83–0.98)	0.01	0.93 (0.85–1.01)	0.09	0.93 (0.85–1.01)	0.09	0.43	0.300	0.265	0.84 (0.76–0.93)	0.001	0.85 (0.75–0.95)	0.005	0.85 (0.75–0.95)	0.005	0.61
Indel-19	2R/2R	447	482								302	250							
	2R/3R	1389	1413								971	754							
	3R/3R	1084	1156		0.62*						752	622		0.64*					
	2R	0.391	0.390								0.389	0.386							
	3R	0.609	0.610	1.01 (0.93–1.08)	0.88	1.02 (0.94–1.10)	0.70	1.02 (0.94–1.10)	0.70	0.80	0.611	0.614	1.01 (0.92–1.11)	0.77	1.03 (0.93–1.14)	0.60	1.03 (0.93–1.14)	0.60	0.84
SNP-63	C/C	2483	2557								1742	1362							
	C/T	402	470								253	234							
	T/T	21	29		0.14*						15	15		0.19*					
	C	0.924	0.914								0.930	0.918							
	T	0.076	0.086	1.14 (1.00–1.30)	0.05	1.06 (0.92–1.22)	0.44	1.06 (0.92–1.22)	0.43	0.44	0.070	0.082	1.18 (0.99–1.40)	0.06	1.08 (0.89–1.32)	0.42	1.08 (0.89–1.32)	0.42	0.41

The UK-1 patient group has been excluded as described in the text. The number of studies for the meta-analysis are as follows: 11 in the overall and 8 in the population-based cases. In the pooled data, all genotypic distributions were in HWE. *P*-values were calculated by genotype or allele. Heterogeneity was considered to be significant if $P < 0.10$ by the Q statistic. Ctrl, controls; T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; Het, heterogeneity.

* *P*-value for comparison of genotype distribution between cases and controls.

calculations for meta-analyses are controversial and were not carried out [25,26].

We first examined each polymorphism individually including testing each for deviation from HWE as well as association with T2D. The UK-1 cases ($n = 153$) displayed a significant departure from HWE at Indel-19 ($P = 0.02$) which led the entire pooled sample to show a significant departure from HWE ($P = 0.04$) and we, thus, excluded this sample (ascertained as a trio sample with living parents) from all further analyses. All of the other case and control groups were in HWE for each variant studied here. In the pooled European sample (excluding the UK-1 cases), the common SNP-43*G allele was associated with increased risk of T2D (OR = 1.11 (1.02–1.20), $P = 0.01$) in the overall data set (Table 2; the OR and P-value were the same if the UK-1 cases were included). The association of the SNP-43*G allele with T2D also approached significance by the meta-analysis (both fixed and random effects models: OR = 1.08 (0.99–1.18), $P = 0.09$) without evidence for heterogeneity ($P = 0.43$).

The less common SNP-63*T allele (allele 2 in the haplotype) was associated with increased risk of T2D: (OR = 1.14 (1.00–1.30), $P = 0.05$) in the pooled analysis of the overall data set (Table 2).

Association of CAPN10 haplotypes with type 2 diabetes

The analysis of the pooled data sets showed that three SNP-43–Indel-19–SNP-63 haplogenotypes were associated with T2D: 1-1-1/2-2-1 (OR = 0.86 (0.75–0.99), $P = 0.03$); 1-1-2/1-2-1 (OR = 1.26 (1.01–1.59), $P = 0.04$); and 1-2-1/1-2-1 (OR = 1.20 (1.03–1.41), $P = 0.02$) (Table 3). The association of the 1-2-1/1-2-1 haplogenotype with T2D was also observed by meta-analysis (fixed effects OR = 1.25 (1.05–1.50), $P = 0.01$; random effects OR = 1.28 (1.03–1.59), $P = 0.02$) but with evidence for heterogeneity ($P = 0.06$) (Table 3; the effect of inclusion of the UK-1 cases on the haplogenotype analysis are described in the legend to Table 3). There was no evidence for publication bias with the 1-2-1/1-2-1 haplogenotype by the standard funnel plot (Begg's adjusted rank correlation test, $P = 0.94$; Egger's regression asymmetry test, $P = 0.27$). However, the power of statistical tests of publication bias are low when there are relatively few studies as in the case of *CAPN10* and T2D. In this case, a nonsignificant result indicates no evidence for substantial bias.

We considered two sources that could be responsible for the observed heterogeneity with respect to the 1-2-1/1-2-1 haplogenotype amongst the data sets: (1) differences in age-at-diagnosis of T2D in different study populations; and (2) the method of ascertainment of cases; i.e., population-based vs. linkage-based. To examine the possible confounding effect of age-at-diagnosis, we split the T2D group based on median age-at-diagnosis (52 years) in the pooled sample and repeated the comparison but using only those cases for whom age-at-diagnosis was available. Splitting on the basis of median age did not resolve the heterogeneity ($P = 0.09$ and 0.11 in younger and elder subsets, respectively). To examine the effect of ascertainment, we examined only studies using cases selected for case-control studies and which we call population-based cases for the purposes of this article although they may not represent a true random sample of cases or controls; i.e., we excluded studies in which the cases were selected from samples used for linkage mapping studies (linkage-based cases): French (probands of sibs), FUSION, UK-2 and Germany-1. Comparing population-based cases ($n = 1627$) and controls ($n = 2027$), removed the heterogeneity with respect to the 1-2-1/1-2-1 haplogenotype ($P = 0.89$) (Table 3 and Fig. 2). Moreover, the strength of the association increased for both the pooled and meta-analyses: OR = 1.46 (1.19–1.78), $P = 0.0003$ and OR = 1.68 (1.33–2.11), $P = 0.00001$, respectively. By contrast, comparing linkage-based cases ($n = 1632$) and controls ($n = 1128$) did not resolve the heterogeneity with respect to the 1-2-1/1-2-1 haplogenotype ($P = 0.07$). There was, moreover, no significant effect of the 1-2-1/1-2-1 haplogenotype on T2D risk in either the pooled (OR = 0.97 (0.77–1.22), $P = 0.80$) or meta-analyses (fixed effects OR = 0.98 (0.76–1.25), $P = 0.85$; random effects OR = 0.98 (0.67–1.43), $P = 0.92$).

We repeated all the analyses using just the population-based cases. The SNP-43*G allele and the 1-2-1 and 2-2-1 haplotypes showed significant association with T2D in both the pooled and meta-analyses (Tables 2 and 4, respectively) and the rare 1-1-2 haplotype reached significance in only the pooled analysis. The haplogenotypes 1-1-2/1-2-1 and 1-2-1/1-2-1 were associated with significantly increased risk and 1-1-1/2-2-1 with reduced risk in the pooled analysis (Table 3). Although the 1-1-2/1-1-2 haplogenotype (Table 3) was also associated with increased risk, it is sufficiently rare that the risk is not significant in sample of this size. Although different inclusion criteria among the controls may also contribute to the heterogeneity, the results suggest that population-based cases may have a different genetic background from linkage-based cases [27]. In this regard, combining the population-based and linkage-based cases contributed a large amount to the observed heterogeneity (62% of the Q statistic).

Haplotype structure across the calpain-10 gene region in Europeans

To construct regional LD context in which to place SNP-43, Indel-19 and SNP-63, we typed 30 polymorphic markers with a mean intermarker distance of 8.8 kb (range = 0.011–50.7 kb) across the *CAPN10* region in a panel of 72 unrelated healthy Americans of European ancestry from San Francisco, California. These markers, selected prior to knowledge of the patterns of variation in the CEPH Europeans in the HapMap, were chosen based on informativeness (minor allele frequency (MAF) $\geqslant 0.05$ and different LD patterns) in our original studies in Mexican Americans [4] and physical coverage of the region. Analysis of the patterns of LD revealed two regions of

Table 3
CAPN10 haplogenotype and risk of type 2 diabetes in Europeans stratified based on ascertainment of cases (population-based vs. linkage-based)

Haplogenotype	Overall									Population-based cases								
	n		Pooled		Meta-analysis					*n*		Pooled		Meta-analysis				
					Fixed effects		Random effects		Het					Fixed effects		Random effects		Het
	Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*	Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*
1-1-1/1-1-1	283	301	1.02 (0.86–1.22)	0.79	1.02 (0.84–1.23)	0.86	1.02 (0.84–1.23)	0.86	0.87	195	162	1.04 (0.84–1.30)	0.71	1.04 (0.82–1.33)	0.74	1.04 (0.82–1.33)	0.74	0.73
1-1-1/1-2-1	580	580	0.95 (0.84–1.08)	0.44	0.94 (0.82–1.08)	0.39	0.94 (0.82–1.08)	0.39	0.99	404	311	0.95 (0.81–1.12)	0.56	0.93 (0.77–1.11)	0.42	0.93 (0.77–1.11)	0.42	0.99
1-1-1/1-1-2	134	146	1.05 (0.82–1.33)	0.70	1.03 (0.80–1.08)	0.81	1.03 (0.80–1.33)	0.81	0.65	90	74	1.03 (0.75–1.41)	0.86	1.03 (0.73–1.44)	0.88	1.03 (0.73–1.44)	0.88	0.43
1-1-1/2-2-1	527	485	0.86 (0.75–0.99)	0.03	0.93 (0.80–1.09)	0.37	0.93 (0.80–1.09)	0.37	0.53	391	269	0.83 (0.70–0.99)	0.03	0.90 (0.73–1.09)	0.28	0.90 (0.73–1.09)	0.28	0.32
1-1-2/1-1-2	19	26	1.32 (0.73–2.38)	0.36	1.09 (0.54–2.21)	0.81	1.09 (0.54–2.21)	0.81	0.35	13	13	1.25 (0.58–2.70)	0.57	0.93 (0.35–2.52)	0.89	0.93 (0.35–2.52)	0.89	0.90
1-1-2/1-2-1	137	178	1.26 (1.01–1.59)	0.04	1.09 (0.84–1.40)	0.51	1.09 (0.84–1.40)	0.51	0.60	78	93	1.52 (1.12–2.06)	0.007	1.31 (0.93–1.87)	0.13	1.31 (0.93–1.87)	0.13	0.85
1-1-2/2-2-1	118	134	1.10 (0.85–1.41)	0.48	1.02 (0.77–1.35)	0.90	1.02 (0.77–1.35)	0.90	0.86	79	64	1.01 (0.72–1.42)	0.94	0.91 (0.62–1.34)	0.64	0.91 (0.62–1.34)	0.64	0.82
1-2-1/1-2-1	317	388	1.20 (1.03–1.41)	0.02	1.25 (1.05–1.50)	0.01	1.28 (1.03–1.59)	0.02	0.06	202	225	1.46 (1.19–1.78)	0.0003	1.68 (1.33–2.11)	0.00001	1.68 (1.33–2.11)	0.00001	0.89
1-2-1/2-2-1	519	533	0.98 (0.86–1.12)	0.81	0.96 (0.83–1.12)	0.63	0.96 (0.83–1.12)	0.63	0.64	365	265	0.89 (0.75–1.06)	0.18	0.85 (0.70–1.03)	0.09	0.85 (0.70–1.03)	0.09	0.86
2-2-1/2-2-1	238	219	0.87 (0.72–1.06)	0.17	0.87 (0.71–1.08)	0.21	0.87 (0.71–1.08)	0.21	0.98	178	121	0.84 (0.66–1.06)	0.15	0.82 (0.63–1.08)	0.16	0.82 (0.63–1.08)	0.16	0.99

The haplotypes are those defined by SNP-43, Indel-19 and SNP-63 and the specific alleles are: SNP-43, allele 1, G and allele 2, A; Indel-19, allele 1, 2 repeats of 32-bp sequence, and allele 2, 3 repeats; and SNP-63, allele 1, C, and allele 2, T. The number of studies is 11 for the meta-analysis in the overall group except 1-1-2/1-1-2 ($n = 6$) and 1-1-2/1-2-1 ($n = 10$) haplogenotypes. The number of studies is 8 for the meta-analysis of the studies using only population-based cases except 1-1-2/1-1-2 ($n = 4$) and 1-1-2/1-2-1 ($n = 7$) haplogenotypes. Heterogeneity was considered to be significant if $P < 0.10$ by the Q statistic. Ctrl, controls; T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; Het, heterogeneity. Note that the results were similar in the overall analysis if the UK-1 cases were included except that the effect of the 1-1-2/1-2-1 haplogenotype was no longer significant (OR = 1.25 (0.99–1.56), $P = 0.06$) in the pooled analysis, and in the meta-analysis, the heterogeneity in the 1-2-1/1-2-1 haplogenotype group was slightly strengthened ($P = 0.05$). In the meta-analysis, the *P*-values for heterogeneity in the 1-2-1/1-2-1 haplogenotype group in population-based and linkage-based cases were 0.89 (see above) and 0.07, respectively.

Table 4
CAPN10 haplogenotype and risk of type 2 diabetes in Europeans stratified based on ascertainment of cases (population-based vs. linkage-based)

Haplotype	Overall									Population-based cases								
	Frequency		Pooled		Meta-analysis					Frequency		Pooled		Meta-analysis				
					Fixed effects		Random effects		Het					Fixed effects		Random effects		Het
	Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*	Ctrl	T2D	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*
1-1-1	0.315	0.303	0.95 (0.88–1.03)	0.18	0.97 (0.88–1.05)	0.42	0.97 (0.88–1.05)	0.42	0.88	0.320	0.306	0.94 (0.85–1.04)	0.23	0.95 (0.85–1.07)	0.42	0.95 (0.85–1.07)	0.42	0.97
1-2-1	0.326	0.346	1.09 (1.01–1.18)	0.02	1.09 (1.00–1.18)	0.06	1.09 (1.00–1.18)	0.06	0.28	0.314	0.350	1.18 (1.07–1.30)	0.001	1.20 (1.08–1.34)	0.001	1.20 (1.08–1.34)	0.001	0.94
1-1-2	0.074	0.085	1.16 (1.02–1.33)	0.03	1.08 (0.93–1.25)	0.32	1.08 (0.93–1.25)	0.32	0.56	0.068	0.080	1.19 (1.00–1.42)	0.05	1.09 (0.89–1.33)	0.41	1.09 (0.89–1.33)	0.41	0.48
2-2-1	0.286	0.266	0.91 (0.84–0.98)	0.02	0.92 (0.84–1.01)	0.07	0.92 (0.84–1.01)	0.07	0.38	0.298	0.263	0.84 (0.76–0.93)	0.0009	0.83 (0.74–0.94)	0.002	0.83 (0.74–0.94)	0.002	0.58

The number of studies is 11 for the meta-analysis in the overall group. The number of studies is 8 for the meta-analysis using population-based cases. Ctrl, controls; T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; Het, heterogeneity.

strong LD or haplotype blocks as defined by Gabriel et al. (D′ confidence intervals) [18] and Wang (four-gamete rule) [19] (Fig. 1). The first spans a region of 36.9 kb (nt −7235 (relative to the ATG of *CAPN10*; SNP-66) to nt 29669 (SNP-32)) following the four-gamete rule, or 39.3 kb (nt −7235 to nt 32076 (SNP-42)) following the D′ confidence interval definition, that includes *CAPN10* as well as the four polymorphisms that have been examined in many studies of this locus (SNP-44, SNP-43, Indel-19 and SNP-63). The second region defined by the four-gamete rule spans 9 kb and is located on the 3′-side of *GPR35* (nt 45632 (SNP-35) to nt 54708 (SNP-31)). Near identical LD patterns are observed in Europeans in HapMap [21]. As we observed in the Mexican American population [28], the LD decays rapidly on both sides of the *CAPN10*-associated haplotype block reducing the likelihood that association between a variant in *CAPN10* and T2D is due to LD with a marker outside this block. This is confirmed by the observation that in the HapMap Europeans [21] no polymorphism within the 5 Mb telomeric end of chromosome 2, other than those within the *CAPN10* block itself which is 1.3 Mb from the telomeric end of chromosome 2, have r^2 values $\geqslant 0.3$

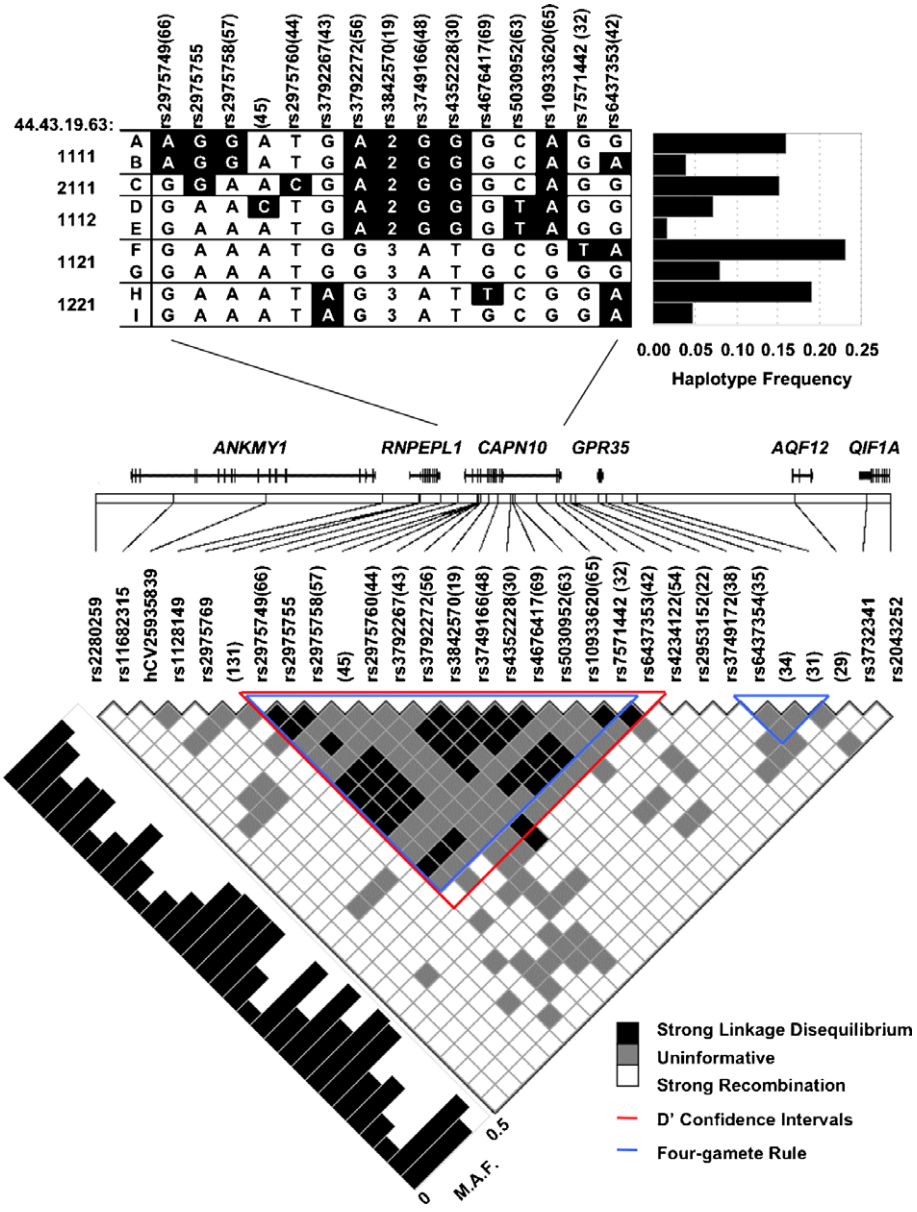

Fig. 1. Linkage disequilibrium and haplotype block structure for the region surrounding *CAPN10* in Europeans. Informative pairwise measures indicating evidence for strong linkage disequilibrium (black), or historical recombination (white) are color-coded in the triangular matrix. Inconclusive pairwise comparisons are grey. Above the LD matrix, polymorphisms (SNP or Indel) are labeled by their dbSNP reference (rs) number followed by their UCSNP designation (in brackets) from the original study [4]. The position of each polymorphism and the refSeq genes mapped within the 255 kb region of 2q37 analysed are also labeled along the diagonal. To the left of the matrix are the minor allele frequencies of each polymorphism. The red line identifies the haplotype block using the D′ confidence intervals [18], and the blue line indicates haplotype blocks following the four-gamete rule [19]. The haplotype frequencies ($\geqslant 1\%$) of the D′ confidence interval defined block are shown at the top. Minor alleles are indicated in black squares, major alleles in white squares, and the haplotype frequencies are noted to the right. Haplotypes are sorted by SNP-44, SNP-43, Indel-19 and SNP-63 polymorphisms.

with SNP-44, -43 or -48 (used as a proxy for Indel-19 since $r^2 = 1$ between SNP-48 and Indel-19 in both the Europeans and Mexican Americans). SNP-63 could not be tested since neither it nor a SNP highly correlated with it is included in the HapMap.

We next calculated the haplotype frequencies by first phasing the genotypes for the 15 polymorphisms in the *CAPN10* block in 63 individuals with genotypes for at least 12 of the 15 polymorphisms. The block is comprised of 6 haplotypes of $\geqslant 5\%$ frequency that together capture 93% of the haplotypic variation at this locus (9 haplotypes $\geqslant 1\%$ account for 98% of the variation). We are confident these 6 haplotypes estimated to occur at a frequency of $\geqslant 5\%$ are "real" since they are each observed in homozygous form in at least one of the 63 individuals. What is quite clear from this pattern of haplotype diversity is that SNP-44, -43, and -63, and Indel-19 are not sufficient to tag all of the variation observed in the block. Forcing these four polymorphisms to tag the remaining eleven polymorphisms, only 4/11 are tagged at $r^2 \geqslant 0.8$ and 8/11 are tagged at $r^2 \geqslant 0.5$. The r^2 threshold must be reduced to 0.223 for SNP-44, SNP-43, Indel-19 and SNP-63 to successfully tag all 11 SNPs in the LD block. Restricting our analyses to common polymorphisms (MAF $\geqslant 0.10$), eight polymorphisms (SNP-66, rs2975755, SNP-44, SNP-43, Indel-19, SNP-69, SNP-32 and SNP-42; SNP-63 was not considered because MAF = 0.068) are required to tag the diversity in this region at $r^2 \geqslant 0.8$ and five polymorphisms (SNP-66, SNP-44, SNP-43, Indel-19 and SNP-32) at $r^2 \geqslant 0.5$. At the same MAF $\geqslant 0.10$ threshold, nine SNPs are required to tag 21 SNPs in HapMap spanning the same region (SNP-66 to SNP-42) at $r^2 \geqslant 0.8$ including rs2953173, SNP-44, SNP-43, SNP-77, Indel-19, SNP-47, rs2953161, rs2953147 and SNP-32.

Discussion

The results of pooled and meta-analyses of 3084 cases (excluding UK-1) and 2935 controls presented here show that genetic variation in *CAPN10* is associated with T2D in Europeans as suggested previously in some studies involving smaller numbers of cases and controls [4,10,11] but not others [6,7,9]. The results are also in agreement with the meta-analysis of Song et al. [13]; however, here we have removed the duplication in some of the data sets examined in their report. The strength of the disease association is similar for SNP-43 and the SNP-43–Indel-19–SNP-63 haplotypes 1-2-1 and 1-1-2 (Tables 2 and 4) consistent with SNP-43 playing role in determining risk as suggested previously [4]. However, not all haplotypes harboring the SNP-43*G allele are associated with increased risk; e.g., 1-1-1. With regard to the 1-1-1 haplotype, SNP-44 defines a subset of haplotypes associated with increased risk and this SNP is itself an independent risk factor for T2D [12]. Thus, haplotypes better describe risk than a single SNP, consistent with either cis-interaction or the tagging of a rarer variant. Since two different haplotypes are associated with increased risk, the former seems more likely than the latter.

In Europeans, haplogenotypes containing both 1-1-2 and 1-2-1 haplotypes are associated with highest risk including the 1-1-2/1-2-1 combination found to be associated with T2D in Mexican Americans although in Mexican Americans neither homozygous combination appeared to be associated with increased risk. The 95% confidence intervals for the odds ratios associated with the homozygous haplogenotypes do, however, overlap in the Europeans and Mexican Americans suggesting that they could have similar effects on risk in both populations. In a Japanese study involving 827 cases and 825 controls, we found no association of the *CAPN10* variants described here with T2D including the 1-2-1/1-2-1 haplogenotype [29]. Moreover, there is no overlap in the 95% confidence interval for 1-2-1/1-2-1 odds ratio in Japanese and Europeans raising the possibility there is additional variation that differentiates risk and non-risk forms of the 1-2-1 haplotype. Such heterogeneity might have contributed to the low risk associated with the 1-2-1 haplotype in the studies in Mexican Americans [4]. Our studies and those of others indicate that the three variants examined here (SNP-43, Indel-19 and SNP-63) and the SNP-44/Thr504Ala polymorphism do not capture all of the variation at *CAPN10* that may be relevant to risk of T2D and related phenotypes. It is also possible that the heterogeneity in risks for the 1-2-1 haplotype observed between European and Japanese samples is attributable to unlinked genetic variants or environmental factors that modify the effects of *CAPN10*.

The results presented here suggest that the method by which cases are selected may confound the results of a case-control study. Our data suggest that cases selected from a previous linkage study (e.g., an affected sibpair study) may differ with respect to genetic risk factors compared to population-based cases although as shown in Fig. 2, the confidence intervals for the OR overlap implying that differences are not significant, at least with these samples. However, the sizes of the groups studied are still relatively small and the differences may disappear when larger numbers of population- and linkage-based cases are examined. *CAPN10* was also identified as a diabetes-susceptibility gene by positional cloning and thus is not a typical candidate gene like *PPARG* and *KCNJ11* which show no evidence for linkage with T2D. In general, we would expect cases from families with at least two affected siblings to generate higher OR for genotypes increasing risk of disease than cases unselected for family history [27]. However, when there are many genetic risk factors that can affect susceptibility, the evidence for linkage can impact the expected OR for a susceptibility locus in that region. For example, we expect the OR for a susceptibility locus estimated from cases drawn from families generating strong evidence for linkage at the location of the susceptibility locus to be higher than the OR in a set of cases unselected for positive family history or linkage evidence. The higher expectation reflects both the general tendency for cases with positive family history (e.g., at least one affected sib) to be enriched for alleles increasing risk of disease and the effects of the "winner's curse" in linkage

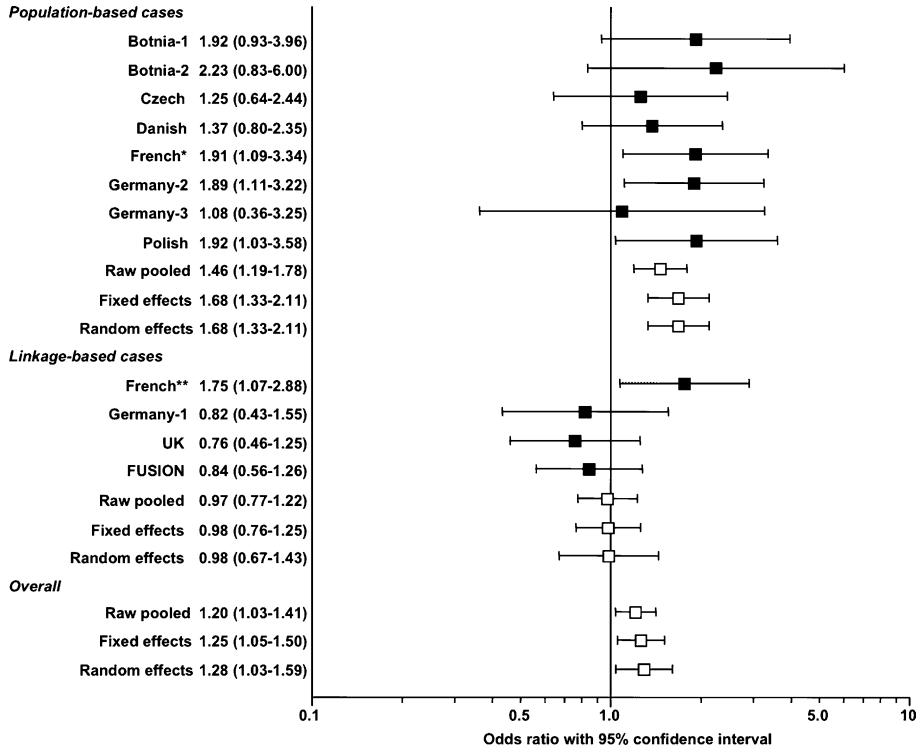

Fig. 2. Meta-analysis plot. The odds ratio with 95% CI for the association of the 1-2-1/1-2-1 haplogenotype with T2D for the 12 studies described here are shown. *The result was derived from French data set excluding probands of sibs. **The result was derived from French data set including probands of sibs. Note that the size of the plotting symbol does not indicate the weight given to the trial in the analysis.

and positional cloning studies. The regions most likely to be subject to positional cloning studies are those with the strongest evidence for linkage. The strongest linkage signals, in turn, overestimate the actual effect size of the susceptibility gene at that location. To the extent that this overestimate reflects a chance oversampling (in the linkage sample) of families segregating for a susceptibility allele at this location, the OR estimates from these same cases will be substantially larger than the OR estimated from cases unselected for linkage to this region. In addition for some genetic models, family history positive but linkage discordant datasets can generate a lower OR than observed in unselected case samples (B. Voight and NJC, unpublished). There may be other explanations for the heterogeneity in OR among the different samples including overall sample size and differences in the ascertainment of controls (see Table 1).

What is the magnitude of the effect of variation in *CAPN10* on T2D risk in Europeans? The results presented here show the effect depends on the combination of specific alleles or haplotypes inherited with some variants increasing risk by perhaps as much as 168% (e.g., meta-analysis and 1-2-1/1-2-1 haplogenotype, Table 3) and other combinations decreasing risk by up to 15% (e.g., SNP-43∗A allele acting in a dominant manner, Table 2). Overall, the effects of variants in *CAPN10* on T2D risk appear similar to those reported for *PPARG* and *KCNJ11*, two generally accepted T2D susceptibility loci, in similar large studies of Europeans [30,31]. The estimates of the effect of *CAPN10* on T2D risk in Europeans are less than noted in our original report which overestimated the effects of this locus (OR for SNP-43 and 1-1-2/1-2-1 haplogenotype = 1.54 and 2.80, respectively) [4]. This may be because we studied cases from families showing evidence for linkage with *CAPN10* or may reflect the instability of point estimates with wide confidence intervals due to small sizes.

The genetic evidence suggests that variation in *CAPN10* affects risk of T2D in Europeans. Variants in *CAPN10* (SNP-44 and SNP-43) and *PPARG* (Pro12Ala mutation) predict T2D in the Botnia study from Finland [32]. Future studies need to define the nature of the cis-interactions in *CAPN10* that distinguish haplotypes that increase risk from those that decrease and determine the best combinations of polymorphisms for defining risk alleles for prospective studies, including efforts using genetic markers to predict future T2D.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2006.05.013.

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