Letter to the Editor

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Meta-Analysis and a Large Association Study Confirm a Role for Calpain-10 Variation in Type 2 Diabetes Susceptibility

To the Editor:

Variation in the calpain-10 gene (*CAPN10* [MIM 605286]) was recently linked and associated with type 2 diabetes mellitus (T2DM) susceptibility (Horikawa et al. 2000). The initial linkage of T2DM to chromosome 2 was found in a population of Mexican Americans from Starr County, Texas (Hanis et al. 1996). Specific combinations of three intronic variants, designated "SNP-43," "SNP-19," and "SNP-63," that capture most of the haplotype diversity at *CAPN10* were associated with a three-fold increased risk of T2DM in this population and could account for the observed linkage (Horikawa et al. 2000). Subsequent association and linkage studies of these three polymorphisms in other populations have produced conflicting results, with association being observed in some populations (Baier et al. 2000 [Pima Indian]; Cassell et al. 2002 [South Indian]; Garant et al. 2002 [African American]; Malecki et al. 2002 [Polish]; Orho-Melander et al. 2002 [Finnish/Botnia]), but not others (Evans et al. 2001 [British]; Hegele et al. 2001 [Oji-Cree Indians]; Tsai et al. 2001 [Samoan]; Xiang et al. 2001 [Chinese]; Daimon et al. 2002 [Japanese]; Elbein et al. 2002 [whites from Utah]; Fingerlin et al. 2002 [Finnish]; Rasmussen et al. 2002 [Danish and Swedish]; Horikawa et al. 2003 [Japanese]).

We previously reported that another variant, SNP-44 (designated "*CAPN10*-g4841T→C"; minor allele frequency 16%), located in intron 3 and 11 bp from SNP-43, was independently associated with T2DM in whites from the United Kingdom (Evans et al. 2001). Further studies have provided tentative support for a role of SNP-44 in T2DM and related traits: associations with polycystic ovary syndrome (Gonzalez et al. 2002) and with measures of oral glucose tolerance (Wang et al. 2002; Tschritter et al. 2003) have been reported. Functional studies suggest that SNP-44 is located in an enhancer element and might affect *CAPN10* expression (Horikawa et al. 2000). Also, in the U.K., German, Japanese, and South Indian populations, SNP-44 is in perfect linkage disequilibrium ($r^2 = 1$) with a missense mutation Thr504Ala (SNP-110) and two polymorphisms in the 5′-UTR (SNP-134 and SNP-135) (Evans et al. 2001; Cassell et al. 2002; Y. Horikawa and P. E. Schwarz, unpublished data).

To assess the association of SNP-44 with T2DM more comprehensively, we performed a meta-analysis of all published SNP-44/T2DM association study data. To identify all relevant published studies, we searched PubMed using the keywords "calpain 10," "diabetes," "44," "SNP 44," "CAPN10," and "type 2," in different combinations. When necessary, authors were contacted to obtain exact genotype numbers, so that precise odds ratios (ORs) from each study could be calculated. Our search identified 10 published case/control studies, consisting of 3,303 subjects. The studies were spread across a number of ethnic groups: British (three studies, Evans et al. 2001); Chinese (Wang et al. 2002); Japanese (Daimon et al. 2002; Horikawa et al. 2003); Finnish/Botnia (two studies, Orho-Melander et al. 2002); South Indian (Cassell et al. 2002); and Mexican American (Horikawa et al. 2000). The frequency of the T2DM-associated SNP-44 C allele (allele 2) ranged from 6% in Mexican Americans to 25% in the Botnia I control population. There was no evidence for OR heterogeneity (Q test $P = .27$), and, although these studies are only a small sample from the many existing T2DM genetic resources, a funnel-plot analysis (Egger et al. 1997) suggested an absence of publication bias ($P = .44$). A Mantel-Haenszel meta-analysis of these studies showed that the C allele was associated with increased risk of T2DM (OR 1.17 [1.02–1.34], $P = .02$).

Three transmission/disequilibrium tests (TDT) had been performed (Evans et al. 2001; Cassell et al. 2002; Orho-Melander et al. 2002). The combined TDT results demonstrated that the C allele was significantly overtransmitted (117 transmitted vs. 77 not transmitted, $P = .004$) from heterozygous parents to diabetic offspring. Although this result cannot be considered independent replication, as proband data was included in the case/control meta-analysis from two of the TDT studies (Evans et al. 2001; Cassell et al. 2002), it provides evidence that the association is not due to population stratification. Of the 10 studies in the meta-analysis, only 1 reported a significant ($P < .05$) association (Evans et al. 2001). However, these studies were small and the

Table 1

Clinical Characteristics of Subjects in Study

	Finding in Study															
	UK4				German1		German2		Czech		Japanese3		Japanese4		Mexican (Mestizo)	
Characteristic	Control EFS[a]	Control ECACC[b]	W2 T2D[c] Probands	YT2D[d]	Control	T2DM	Control	T2DM	Control	T2DM	Control	T2DM	Control	T2DM	Control	T2DM
SNP-44 minor allele frequency	.16	.16	.20	.19	.11	.14	.16	.17	.l2	.14	.09	.06	.12	.14	.04	.09
N [% males]	994 [50]	335 [56]	399 [54]	297 [55]	73 [35]	308 [38]	235 [56]	244 [59]	110 [69]	279 [36]	206 [67]	206 [59]	90 [33]	189 [57]	114 [50]	134 [42]
Average age ± SD (years)	32.5 ± 5.5	NA	70.6 ± 8.6	NA	50.8 ± 11.9	61.8 ± 11.3	65 ± 5.4	65.0 ± 5.1	18.1 ± 2.3	58.5 ± 7.4	67.9 ± 5.5	59.1 ± 13.0	68.2 ± 5.8	61.7 ± 12.8	49.9 ± 14.4	55.1 ± 9.8
Age at diagnosis ± SD (years)	NA	NA	55.2 ± 8.5	40.5 ± 10.0	NA	49.2 ± 12.4	NA	NA	NA	49.6 ± 8.7	NA	45.8 ± 12.8	NA	50.3 ± 12.9	NA	44.8 ± 7.5
BMI (kg/m^2) ± SD	26.7 ± 3.8[e]	NA	28.9 ± 5.4	30.9 ± 4.5	24.9 ± 4.4	28.7 ± 4.8	27.5 ± 3.8	31.0 ± 4.7	24.3 ± 4.0	30.1 ± 5.3	23.0 ± 2.5	23.5 ± 3.6	23.0 ± 2.9	23.7 ± 4.7	26.5 ± 3.8	27.4 ± 4.1
% Receiving treatment:																
Diet	NA	NA	16	9	NA	13	NA	56	NA	26	NA	20	NA	12	NA	NA
OHA[f]	NA	NA	70	38	NA	33	NA	28	NA	58	NA	40	NA	53	NA	NA
Insulin	NA	NA	14	53	NA	53	NA	16	NA	16	NA	40	NA	35	NA	NA

Note.—Continuous variables are presented as mean ± SD. NA = not applicable or not available.

[a] EFS = Exeter Family Study.

[b] ECACC = European Collection of Cell Cultures.

[c] W2 T2D = Warren 2 Type 2 Diabetes Collection.

[d] YT2D = Young-Onset Type 2 Diabetes Collection.

[e] Males only, as females were pregnant.

[f] OHA = oral hypoglycemia agents.

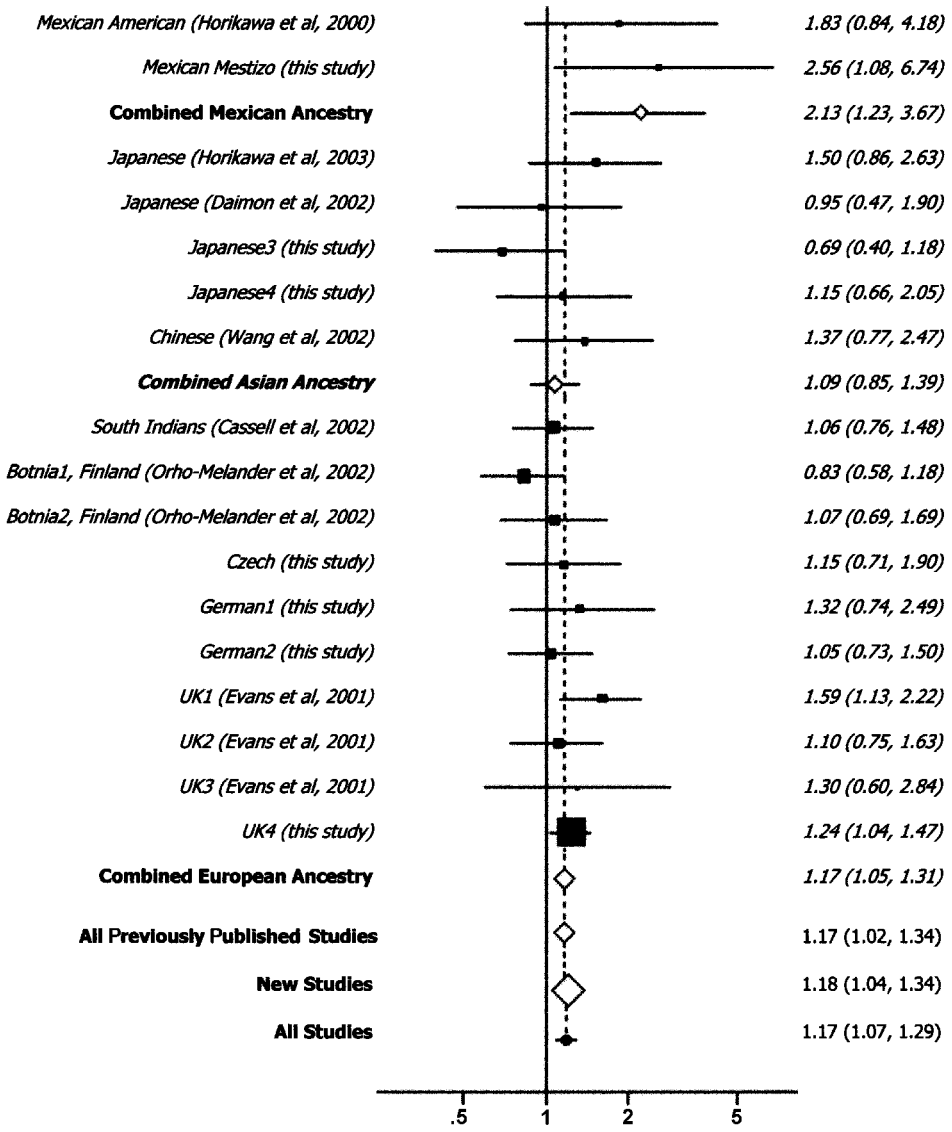

Figure 1 Mantel-Haenszel OR meta-analysis plot (fixed effects) for SNP-44 association with T2DM. Point estimates and 95% CLs for each previously published, new, and combined case/control study.

mean power to detect an OR of 1.17 at $P < .05$ was ~11% (range 5%–14%).

In the context of genetic association studies, which test many polymorphisms in numerous candidate genes, a P value of .02 can only be considered evidence suggestive of a real association. We therefore genotyped SNP-44 in an additional 4,213 subjects: 3,274 white European subjects from four case/control studies (one British, two German, and one Czech); 691 Japanese subjects from two case/control studies; and 248 Mexican (mestizo) subjects from Mexico City and Orizaba City from one case/control study. Overall, this provided 2,056 subjects with T2DM and 2,157 controls, and a power of ~80% to detect an OR of 1.17. Clinical details of the study subjects are presented in table 1; further details are available as supplementary information from the authors. All studies were approved by the relevant ethics committee, and all subjects gave their informed consent.

When all the studies were combined, there was no evidence for between-studies OR heterogeneity (Q test $P = .23$); a Mantel-Haenszel fixed-effects model was therefore used for subsequent analysis. Meta-analysis of the new studies gave an OR for the SNP-44 C allele of 1.18 (1.04–1.34), $P = .01$ (fig. 1). A combined meta-analysis of all previously published data and our new data gave an OR of 1.17 (1.07–1.29), $P = .0007$. All study populations were in Hardy-Weinberg equilibrium except the T2DM cohort of Horikawa et al. 2003 ($P = .005$) and the control population of the third Jap-

anese study ($P = .02$). Although these deviations may be due to random fluctuation and multiple-hypothesis testing, they contributed a large amount to heterogeneity (27% of the Q statistic); excluding these studies, the SNP-44 C allele OR for the new studies was 1.23 (1.07–1.40), $P = .003$; the overall OR was 1.19 (1.08–1.31), $P = .0005$. This OR is of similar magnitude to that of E23K (Gloyn et al. 2003; Love-Gregory et al. 2003; Nielsen et al. 2003) and Pro12Ala (Altshuler et al. 2000), the other common variants confirmed as T2DM-susceptibility polymorphisms. An OR of 1.17 is low and may help explain why there is little evidence for linkage of the *CAPN10* region to T2DM in most populations. The haplotypes responsible for the *CAPN10* linkage seen in the Mexican American population were associated with a higher T2DM OR (~3.0) and were more likely to be detected by linkage analysis (Horikawa et al. 2000). These haplotypes are less common in other populations.

SNP-44 is in perfect linkage disequilibrium ($r^2 = 1$) with the missense mutation, Thr504Ala, and two SNPs (SNP-134 and SNP-135) in the 5′-UTR and therefore may not be the causal variant. Further haplotype and functional analyses are required to confirm which of these polymorphisms contribute to T2DM susceptibility.

In conclusion, our results have confirmed that a *CAPN10* haplotype defined by the SNP-44 polymorphism predisposes to T2DM. Meta-analyses of published genetic associations, combined with large replication studies, are a powerful approach to detecting susceptibility variants in common disease.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for calpain-10, KCNJ11, and PPARγ)

PubMed, http://www.ncbi.nlm.nih.gov/PubMed/

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