

Variants of the Transcription Factor 7-Like 2 (TCF7L2) Gene Are Associated With Type 2 Diabetes in an African-American Population Enriched for Nephropathy

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OBJECTIVE—Recently, variants in the TCF7L2 gene have been reported to be associated with type 2 diabetes across multiple European populations, but only one small sample of African-American type 2 diabetic patients has been examined. Our objective was to investigate the importance of TCF7L2 in a larger African-American case-control population.

RESEARCH DESIGN AND METHODS—We investigated single nucleotide polymorphisms (SNPs) in six known type 2 diabetes genes in 577 African-American case subjects with type 2 diabetes enriched for nephropathy and 596 African-American control subjects. Additionally, we genotyped 70 ancestry-informative markers (AIMs) to apply adjustments for differences in ancestral proportions.

RESULTS—The most significant associations were observed with TCF7L2 intron 3 SNPs rs7903146 (additive $P = 4.10 \times 10^{-6}$, odds ratio [OR] 1.51; admixture-adjusted $P_a = 3.77 \times 10^{-6}$) and rs7901695 ($P = 0.001$, OR 1.30; $P_a = 0.003$). The 2-SNP haplotype containing these SNPs was also associated with type 2 diabetes ($P = 3 \times 10^{-5}$). Modest associations were also seen with TCF7L2 intron 4 SNPs rs7895340, rs11196205, and rs12255372 ($0.01 < P < 0.05$; $0.03 < P_a < 0.08$), as well as with ATP-sensitive inwardly rectifying potassium channel subunit Kir6.2 (KCNJ11) and hepatocyte nuclear factor 4- α (HNF4A) SNPs ($0.01 < P < 0.05$; $0.01 < P_a < 0.41$). No significant associations were detected with genotyped calpain 10 (CAPN10), peroxisome proliferator-activated receptor γ (PPARG), and transcription factor 1 (TCF1) SNPs.

CONCLUSIONS—This study indicates that variants in the TCF7L2 gene significantly contribute to diabetes susceptibility in African-American populations. *Diabetes* 56:2638–2642, 2007

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AIM, ancestry-informative marker; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

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Few genetic variants have shown association with type 2 diabetes across populations. These include polymorphisms in calpain 10 (CAPN10) (1), peroxisome proliferator-activated receptor γ (PPARG) (2), ATP-sensitive inwardly rectifying potassium channel subunit Kir6.2 (KCNJ11) (3), hepatocyte nuclear factor 4- α (HNF4A) (4), and hepatic transcription factor 1 (TCF1) (5) genes. Several reports have confirmed associations between intron 3 and intron 4 polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes (6–13).

Three studies of TCF7L2 have included African-American or U.K. Afro-Caribbean individuals. In the Diabetes Prevention Program (14), incident diabetes occurred in 139 of 605 African-American participants, and results for the two TCF7L2 SNPs genotyped (rs12255372 and rs7903146) were not significant in African Americans. A case-control study that included U.K. Europeans, Indian Asians, and Afro-Caribbeans did not find significant heterogeneity between groups, although associations did not reach significance in the Afro-Caribbean group ($n = 385$) (13). A study of 118 nondiabetic female African-American subjects found a significant association between rs12255372 and lower disposition index (15).

To investigate reported TCF7L2 SNPs in a larger African-American type 2 diabetic case-control population and to place results in the context of other type 2 diabetes loci, we genotyped 13 SNPs in six known diabetes genes. Additionally, we estimated ancestral proportions for study subjects using ancestry informative markers (AIMs) to take into account the impact of admixture on association results.

RESEARCH DESIGN AND METHODS

This study was conducted under institutional review board approval from Wake Forest University School of Medicine and adhered to the tenets of the Declaration of Helsinki. Recruitment of African-American and European-American patients and control subjects has previously been described (16). Briefly, 577 unrelated African-American patients with type 2 diabetes, born in North Carolina, South Carolina, Georgia, Tennessee, or Virginia, were recruited from dialysis facilities. Presumed type 1 diabetic patients were excluded on the basis of a history of diabetic ketoacidosis or diabetes diagnosis before age 25 years with continuous insulin therapy since diagnosis. A diagnosis of type 2 diabetes was based on participants reporting an initial diagnosis of diabetes after age 35 years, receiving oral hypoglycemic agents or dietary therapy without insulin for at least 1 year after initial diagnosis, and active treatment with diabetes medications. Case subjects had type 2 diabetes diagnosed at least 5 years before initiating renal replacement therapy, background or greater diabetic retinopathy, and/or $>3+$ proteinuria on urinalysis in the absence of other causes of nephropathy. A total of 596 African-American and 36 European-American control subjects, born in North

TABLE 1
Characteristics of African-American subjects

Trait	Type 2 diabetes–ESRD case subjects		Control subjects	
	<i>n</i> *	Mean ± SD	<i>n</i> *	Mean ± SD
Male/female	225/352	—	292/304	—
Age at exam (years)	541	62.2 ± 10.3	448	49.3 ± 9.8
Age at type 2 diabetes diagnosis (years)	544	41.8 ± 11.6	—	NA
Age at ESRD diagnosis (years)	560	59.0 ± 10.5	—	NA

*Number with data available. All case subjects were diagnosed with type 2 diabetes and ESRD (end-stage renal disease). NA, not applicable.

Carolina, South Carolina, Georgia, Tennessee, or Virginia, without a current diagnosis of type 2 diabetes or renal disease were recruited from community sources. DNA extraction was performed using the PureGene system (Gentra Systems, Minneapolis, MN). DNA was obtained from 44 Yoruba Nigerians from the National Institute of General Medical Sciences Human Variation Collection (Coriell Cell Repositories, Camden, NJ).

Diabetes candidate gene genotyping. SNPs CAPN10 rs3792267; PPARG rs1801282; TCF7L2 rs7901695, rs7903146, rs7895340, rs11196205, and rs12255372; and HNF4A rs1884613 and rs2144908 were genotyped by Illumina's Custom Genotyping Service (San Diego, CA). SNPs genotyped using iPLEX methodology (Sequenom, San Diego, CA) (17) were CAPN10 rs2975760, KCNJ11 rs5219, TCF1 rs1800574, and HNF4A rs4810424. Primer sequences are available on request. CAPN10 SNP rs5030952 (UCSNP-63) was unable to be genotyped successfully on either platform. Genotyping success rates for candidate SNPs were >97.7% in case subjects and >96.5% in control subjects. TCF7L2 SNP genotyping success rates were 100% in case subjects and >99.8% in control subjects. Concordance rates for 50 replicate pairs were 100% for all SNPs except rs4810424, where there were two discordant alleles among 96 chromosomes.

Genotyping for admixture analyses. A total of 70 AIMs were genotyped by Illumina's Custom Genotyping Service or using a MassARRAY system (Sequenom) (17) in 577 African-American case subjects, 596 African-American control subjects, 44 Yoruba Nigerians, and 39 European Americans (Supplementary Table 1 [available in an online appendix at <http://dx.doi.org/10.2337/db07-0012>]). Primer sequences are available on request. Genotyping success rates for AIMs were >97.4% in African-American case subjects and >95.6% in African-American control subjects.

Statistical analyses. Haplotype block structure was established using Haploview 3.2 (18), with the block definition from Gabriel et al. (19). Unadjusted genotypic association tests were assessed by a χ^2 statistic using 2×2 and 2×3 contingency tables.

Tests of haplotypic association were performed using the score test (Haplo.score) implemented in the Haplo.stats package (20). Analyses were initially performed using 1,000 permutations. Where association tests indicated possible significance (empirical global P value < 0.10), permutations were increased (10,000–1,000,000). Haplotypes with <2% overall frequency were removed from analyses. Estimates of case and control haplotype frequencies were obtained using Dandelion 1.26 (21).

Individual ancestral proportions were estimated using an EM algorithm (FRAPPE) (22) under a two-population model. Logistic regression tests of additive (or dominant) genetic models included adjustments for individual estimates of African ancestry (23).

Sequencing. SNPs were tested for departures from Hardy-Weinberg equilibrium (HWE). SNPs that deviated from HWE were sequenced in 23 African-American case and 23 African-American control subjects using BigDye Terminator Cycle Sequencing Kits (version 1.1; Applied Biosystems, Foster City, CA) and a 3730xl DNA Analyzer (Applied Biosystems). Data were viewed using Sequencher (version 4.1.4; Gene Codes Corporation, Ann Arbor, MI).

RESULTS

Population characteristics are shown in Table 1. Control subjects were significantly younger than case subjects ($P < 0.0001$) but older than mean age at type 2 diabetes diagnosis ($P < 0.0001$). Age data were unavailable for 25% of control subjects. BMI data are not presented because measures reflected weight on dialysis in case subjects and were unavailable for the majority of control subjects. A higher proportion of females (61%) was observed in the case than in the control subjects (51%), possibly due to a combination of higher prevalence of type 2 diabetes in women, as well as participation and survival bias.

In African-American case subjects, the mean ± SD proportion of African ancestry was estimated to be 0.817 ± 0.133 , while in African-American control subjects it was 0.791 ± 0.131 . Distributions are shown in Supplementary Fig. 1.

HNF4A SNP rs1884613 was inconsistent with HWE in African-American control subjects ($P = 0.01$). PPARG rs1801282 ($P = 0.007$) and KCNJ11 rs5219 ($P = 0.0009$) deviated from HWE proportions in African-American case subjects most likely because the minor alleles for these SNPs are rare (0.014 and 0.056, respectively) (Table 2). Additionally, rs5219 minor allele frequency estimates for “ancestral” African ($n = 44$) and European-American populations ($n = 39$) are at the extremes (0 in Africans and 0.408 in European Americans), suggesting that genotype frequencies will be greatly influenced by admixture. Two SNPs were inconsistent with HWE in both case and control subjects: 1) TCF7L2 rs7903146, where $P = 0.016$ (due to heterozygote excess) in case subjects and $P = 0.015$ (homozygote excess) in control subjects and 2) HNF4A rs4810424, where $P < 0.0001$ in both case and control subjects, with heterozygotes fewer than expected in both samples. Sequencing results from 23 case and 23 control subjects for these five SNPs were 100% concordant with Illumina or Sequenom calls, except for HNF4A rs4810424, which had one discordant allele call from 92 chromosomes.

Linkage disequilibrium metrics between the two CAPN10 SNPs were $D' = 1.00$ ($r^2 = 0.006$). The TCF7L2 SNPs fell into two blocks of linkage disequilibrium (Fig. 1), with the first block containing intron 3 SNPs rs7901695 and rs7903146 and the second block containing intron 4 SNPs rs7895340, rs11196205, and rs12255372. The three HNF4A SNPs were in high linkage disequilibrium, with pairwise values of $D' = 0.95$ ($r^2 = 0.66$) between rs4810424 and rs1884613, $D' = 0.97$ ($r^2 = 0.85$) between rs1884613 and rs2144908, and $D' = 0.84$ ($r^2 = 0.58$) between rs4810424 and rs2144908.

Single SNP association results are presented in Table 2. Genotype frequencies and counts for each SNP are shown in Supplementary Table 2. The most significant associations were seen with TCF7L2 intron 3 SNPs rs7903146 (additive $P = 4.10 \times 10^{-6}$; admixture-adjusted $P_a = 3.77 \times 10^{-6}$) and rs7901695 ($P = 0.001$; $P_a = 0.003$). The recessive association with TCF7L2 rs7895340 ($P = 0.011$) (Table 2) is based on relatively few homozygous individuals (Supplementary Table 2). Other SNPs that showed modest evidence of association included TCF7L2 intron 4 SNPs rs11196205 and rs12255372; HNF4A SNPs rs4810424, rs1884613, and rs2144908; and KCNJ11 rs5219 (Table 2).

Haplotype analysis of the two CAPN10 SNPs did not exhibit a significant association with type 2 diabetes ($P = 0.96$; data not shown). TCF7L2 haplotype block one, containing rs7901695 and rs7903146, was significantly as-

TABLE 2
Single SNP genotypic tests of association with type 2 diabetes and end-stage renal disease

Gene	SNP	Major/ minor alleles*	Minor allele frequency controls	Minor allele frequency cases	Dominant P^{\dagger}	OR (95% CI)	Additive P	OR (95% CI)	Recessive P	OR (95% CI)	Admixture- adjusted additive P
CAPN10	rs2975760	T/C	0.037	0.036	0.689	0.91 (0.59-1.42)	—	—	—	—	—
CAPN10	rs3792267	G/A	0.155	0.156	0.589	1.07 (0.83-1.38)	0.960	1.01 (0.80-1.26)	0.153	0.57 (0.26-1.24)	—
PPARG	rs1801282	C/G	0.020	0.177	0.185	0.65 (0.34-1.23)	—	—	—	—	—
TCF7L2	rs7901695	T/C	0.455	0.474	0.004	1.47 (1.14-1.91)	0.001	1.30 (1.11-1.53)	0.023	1.37 (1.04-1.79)	0.003
TCF7L2	rs7903146	C/T	0.276	0.366	1.79×10^{-8}	1.95 (1.54-2.46)	4.10×10^{-6}	1.51 (1.27-1.80)	0.444	1.16 (0.79-1.69)	3.77×10^{-6}
TCF7L2	rs7895340	A/G	0.226	0.188	0.100	0.82 (0.65-1.04)	—	—	—	—	0.071
TCF7L2	rs11196205	C/G	0.224	0.186	0.091	0.81 (0.64-1.03)	0.022	0.79 (0.65-0.97)	0.011	0.45 (0.24-0.85)	0.078
TCF7L2	rs12255372	G/T	0.282	0.320	0.020	1.31 (1.04-1.65)	0.026	0.79 (0.65-0.97)	0.022	0.49 (0.26-0.91)	0.037
KCNJ11	rs5219	G/A	0.071	0.056	0.045	0.69 (0.49-0.99)	0.044	1.20 (1.00-1.43)	0.630	1.10 (0.74-1.66)	0.173 \ddagger
TCF1	rs1800574	C/T	0.004	0.004	0.959	1.03 (0.33-3.21)	—	—	—	—	—
HNF4A	rs4810424	C/G	0.107	0.147	0.032	1.39 (1.03-1.88)	0.018	1.28 (1.04-1.57)	0.038	1.63 (1.02-2.61)	0.014
HNF4A	rs1884613	C/G	0.091	0.116	0.019	1.42 (1.06-1.90)	0.042	1.31 (1.01-1.70)	0.837	0.93 (0.37-2.30)	0.026
HNF4A	rs2144908	G/A	0.097	0.126	0.018	1.41 (1.06-1.87)	—	—	—	—	0.408 \ddagger

*Major allele is defined as most common allele in controls. \dagger Only the dominant model was considered where the minor allele homozygote count for either case or control subjects was <10 . Test models refer to the minor allele. \ddagger Dominant model. Boldface: reported risk allele and $P < 0.05$.

sociated with type 2 diabetes ($P = 3 \times 10^{-5}$; empirical $P = 1 \times 10^{-5}$) (Table 3); however, haplotype block 2—containing rs7895340, rs11196205, and rs12255372—did not reach significance ($P = 0.056$; empirical $P = 0.051$; data not shown). The HNF4A 2-SNP haplotype containing rs4810424 and rs1884613 showed nominal evidence of association with type 2 diabetes, as did the 3-SNP haplotype containing all HNF4A SNPs genotyped (Supplementary Table 3).

DISCUSSION

We investigated SNPs in six known type 2 diabetes candidate genes for association with type 2 diabetes in an African-American population. The strongest associations with type 2 diabetes were TCF7L2 intron 3 SNPs rs7901695 and rs7903146. Recently, Helgason et al. (24) reported rs7903146 to be the type 2 diabetes risk variant in West Africans. The frequency of the rs7903146 T (risk)-allele was similar in Africans (0.284), African-American control subjects (0.275), European Americans (0.269), and the previously reported African-American sample (0.31) (14). In contrast, the rs7901695 C (risk)-allele was present at a higher frequency in Africans (0.466), African-American control subjects (0.455), and the Amish (0.40) (10) than in European-American (0.295) and other European populations (0.22–0.30) (6,7) and was the major allele in African-American case subjects (0.501). For rs7903146, there was a significant increase in relative frequency of heterozygotes in case subjects compared with that in control subjects (+15%), with a comparatively small change in TT homozygotes (+1%). Calculations using a range of allele frequencies bounded by 0.276 (control subjects) to 0.366 (case subjects) suggest that the TT genotype relative risk is smaller than that for heterozygotes; hence, risk is best described by an overdominant model. This is also reflected in the increased OR for the dominant (1.95) versus the additive (1.51) test.

The TCF7L2 SNPs fell within two haplotype blocks (Fig. 1), consistent with the low linkage disequilibrium between rs7903146 and rs12255372 in other African-American populations (14,15). It is possible that the higher linkage disequilibrium observed in Europeans has obscured the primary region of association; our results suggest that it may be productive to focus future type 2 diabetes analyses on TCF7L2 intron 3.

Reported associations of type 2 diabetes with SNPs in CAPN10 (25), TCF1 (5), and PPARG (2) were not detected (Table 2). Although nonsignificant, the trend for PPARG P12 risk is in the expected direction and the OR close to reported values (2).

Borderline association with KCNJ11 E23K ($P = 0.044$) (Table 2) is in the direction opposite that in previous reports (3). HNF4A associations with type 2 diabetes (Table 2 and Supplementary Table 3) are in the expected directions (4). However, concordance rates for HNF4A rs4810424 are below 100% for genotyping (97.9%) and sequencing (98.9%), and this SNP shows substantial deviations from HWE estimates ($P < 0.0001$), suggesting that results should be viewed with caution.

Our ascertainment scheme did not distinguish whether associations were with type 2 diabetes or diabetic nephropathy. However, all SNPs in candidate genes were selected on the basis of previous associations with type 2 diabetes. It remains unknown whether TCF7L2 is independently associated with diabetic nephropathy. The majority

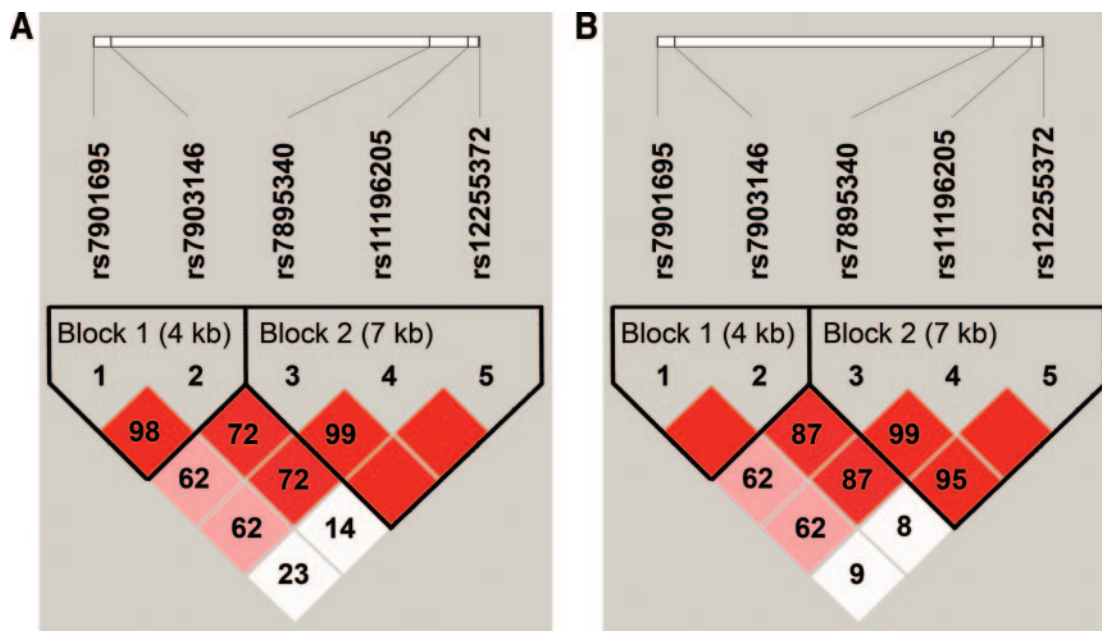


FIG. 1. Linkage disequilibrium structure of the TCF7L2 gene in African-American case ($n = 577$) (A) and control ($n = 596$) (B) subjects, with haplotype blocks based on the definition of Gabriel et al. (19) implemented in Haploview. D' values are displayed in the squares. Empty squares represent a pairwise $D' = 1$; red squares represent high pairwise linkage disequilibrium, coloring down to white squares of low pairwise linkage disequilibrium.

of control subjects were not tested for diabetes, and none were tested for renal impairment; thus, the control sample probably contains a small proportion of undiagnosed type 2 diabetic case subjects and individuals with renal dysfunction. While this has not affected our ability to detect positive associations with TCF7L2, it likely reduced our power to detect more subtle influences of variants in other genes investigated and led to underestimation of ORs.

While adjustment for admixture reduced the strength of the majority of observed associations (Table 2), associations with TCF7L2 SNPs rs7903146 and rs7901695 remained significant. Five self-identified African-American case and four African-American control subjects had estimates of African ancestry below 5%. Removing these individuals from analyses does not substantially change any of our conclusions (data not shown).

This study demonstrates that TCF7L2 is an important diabetes gene in African-American populations. Our results place the strong associations with TCF7L2 SNPs within the context of modest or nonsignificant effects in this population of previously reported type 2 diabetes loci and, more precisely, localize the region associated with disease to intron 3. Further investigations of the mechanism of TCF7L2 risk should be a priority.

TABLE 3

Association analysis for TCF7L2 haplotype containing rs7901695 and rs7903146

Haplo-type	Case frequency	Control frequency	Haplo-type score	P	Empirical P
Global	—	—	—	3×10^{-5}	1×10^{-5}
TC	0.467	0.506	-3.34	0.0009	0.0008
CC	0.169	0.219	-1.37	0.17	0.18
CT	0.345	0.268	4.61	$<1 \times 10^{-5}$	1×10^{-5}

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REFERENCES

- Cox NJ, Hayes MG, Roe CA, Tsuchiya T, Bell GI: Linkage of calpain 10 to type 2 diabetes: the biological rationale. *Diabetes* 53 (Suppl. 1):S19–S25, 2004
- Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
- Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM: Large-scale association studies of variants in genes encoding the pancreatic β -cell K_{ATP} channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572, 2003
- Love-Gregory linkage disequilibrium, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA: A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 α gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140, 2004
- Weedon MN, Owen KR, Shields B, Hitman G, Walker M, McCarthy MI, Hattersley AT, Frayling TM: A large-scale association analysis of common variation of the HNF1 α gene with type 2 diabetes in the U.K. Caucasian population. *Diabetes* 54:2487–2491, 2005
- Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir A, Stykkarsdóttir U, Magnusson KP, Walters GB, Palsdóttir E, Jonsdóttir T, Gudmundsdóttir T, Gylfason A, Saemundsdóttir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdóttir U, Gulcher JR, Kong A, Stefansson K: Variant of transcription

- factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323, 2006
7. Dancott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shuldiner AR: Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55:2654–2659, 2006
 8. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, McCarthy MI: Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 55:2640–2644, 2006
 9. Saxena R, Giannini L, Burt NP, Lyssenko V, Giuducci C, Sjogren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D: Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55:2890–2895, 2006
 10. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Collins FS, Boehnke M: Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. *Diabetes* 55:2649–2653, 2006
 11. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, Scharfmann R, Staels B, Fruhbeck G, Froguel P: Transcription factor TCF7L2 genetic study in the French population: expression in human β -cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 55:2903–2908, 2006
 12. van Vliet-Ostapchouk JV, Shiri-Sverdlov R, Zhernakova A, Strengman E, van Haften TW, Hofker MH, Wijmenga C: Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. *Diabetologia* 50:59–62, 2007
 13. Humphries SE, Gable D, Cooper JA, Ireland H, Stephens JW, Hurel SJ, Li KW, Palmieri J, Miller MA, Cappuccio FP, Elkeles R, Godsland I, Miller GJ, Talmud PJ: Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. *J Mol Med* 84 (Suppl. 12):1–10, 2006
 14. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D: TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241–250, 2006
 15. Munoz J, Lok KH, Gower BA, Fernandez JR, Hunter GR, Lara-Castro C, De Luca M, Garvey WT: Polymorphism in the transcription factor 7-like 2 (TCF7L2) gene is associated with reduced insulin secretion in nondiabetic women. *Diabetes* 55:3630–3634, 2006
 16. Freedman BI, Yu H, Spray BJ, Rich SS, Rothschild CB, Bowden DW: Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. *Kidney Int* 51:819–825, 1997
 17. Buettow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A: High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci U S A* 98:581–584, 2001
 18. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of linkage disequilibrium and haplotype maps. *Bioinformatics* 21:263–265, 2005
 19. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002
 20. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA: Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 70:425–434, 2002
 21. Green LE, Lange EM, Langefeld CD: Power comparisons of phase-known versus phase-unknown haplotype analyses for case-control designs (Abstract). *Am J Hum Genet* 69:1948a, 2001
 22. Tang H, Peng J, Wang P, Risch NJ: Estimation of individual admixture: analytical and study design considerations. *Genet Epidemiol* 28:289–301, 2005
 23. Redden DT, Divers J, Vaughan LK, Tiwari HK, Beasley TM, Fernandez JR, Kimberly RP, Feng R, Padilla MA, Liu N, Miller MB, Allison DB: Regional admixture mapping and structured association testing: conceptual unification and an extensible general linear model. *PLoS Genet* 2:e137, 2006
 24. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, Adeyemo A, Chen Y, Chen G, Reynisdottir I, Benediktsson R, Hinney A, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Schafer H, Faruque M, Doumatey A, Zhou J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Sigurdsson G, Hebebrand J, Pedersen O, Thorsteinsdottir U, Gulcher JR, Kong A, Rotimi C, Stefansson K: Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 39:218–225, 2007
 25. Garant MJ, Kao WH, Brancati F, Coresh J, Rami TM, Hanis CL, Boerwinkle E, Shuldiner AR: SNP43 of CAPN10 and the risk of type 2 diabetes in African-Americans: the Atherosclerosis Risk in Communities Study. *Diabetes* 51:231–237, 2002