Functional Variants in *MBL2* Are Associated With Type 2 Diabetes and Pre-Diabetes Traits in Pima Indians and the Old Order Amish

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OBJECTIVE—*MBL2* encodes the mannose-binding lectin, which is a key player in the innate immune system and has recently been found to play a role in insulin resistance and development of type 1 diabetes and gestational diabetes mellitus. To assess the role of *MBL2* in diabetes susceptibility, this gene was analyzed in the Pima Indian population, which has a high prevalence of type 2 diabetes.

RESEARCH DESIGN AND METHODS—Nineteen tag single nucleotide polymorphisms (SNPs) were genotyped in a population-based sample of 3,501 full-heritage Pima Indians, and selected SNPs were further genotyped in independent samples of Native American (n = 3,723) and Old Order Amish (n = 486) subjects.

RESULTS—Two variants, a promoter SNP (rs11003125) at −550 bp with a risk allele frequency of 0.77 and a Gly54Asp (rs1800450) with a risk allele frequency of 0.83, were associated with type 2 diabetes in the full-heritage Pima Indians (odds ratio 1.30 per copy of the G allele for rs1103125, P = 0.0007, and 1.30 per copy of the glycine allele for rs1800450, P = 0.002, adjusted for age, sex, birth year, and family membership). These associations replicated in an independent Native American sample (1.19, P = 0.04, for rs11003125) and a Caucasian sample, the Old Order Amish (1.51, P = 0.004, for rs1103125 and 2.38, P = 0.003, for rs1800450). Among Pima Indians with normal glucose tolerance, the diabetes risk allele glycine of Gly54Asp was associated with a decreased acute insulin response to an intravenous glucose bolus infusion (P = 0.004, adjusted for age, sex, percent body fat, glucose disposal under physiological insulin stimulation, and family membership).

CONCLUSIONS—Our data suggest that the functional variants in *MBL2* contribute to type 2 diabetes susceptibility in both Native Americans and the Old Order Amish. ***Diabetes*** **59: 2080–2085, 2010**

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Mannose-binding lectin (MBL) is a liver-derived serum lectin involved in the innate immune defense. Upon binding to specific carbohydrate structures on various microorganisms, MBL may utilize MBL serine protease (MASP)-2 to activate the third pathway of complement (lectin pathway) and thereby opsonophagocytosis (1).

Serum MBL levels have been shown to be strongly correlated with the presence of variants within the *MBL2* gene. Missense polymorphisms at codon 54 (resulting in a glycine to aspartic acid), codon 57 (resulting in a glycine to glutamic acid), and codon 52 (resulting in an arginine to cysteine) impair oligomer formation, leading to reduced serum levels of functional MBL. In addition, three promoter polymorphisms at position −550 bp G > C (H/l), −221 bp G > C (Y/X) and +4 bp C > T (P/Q) influence the expression of MBL2 (1–3).

Deficiency of MBL has been associated with immunodeficiency, autoimmune disorders such as systemic lupus erythematosus, and rheumatoid arthritis (4,5). Recent studies have further implicated MBL deficiency in the development of type 1 diabetes (6), gestational diabetes mellitus (7), diabetic nephropathy (8), and insulin resistance and obesity (9). Based on the biological role of MBL2, this gene was investigated as a potential susceptibility gene for type 2 diabetes in Pima Indians.

RESEARCH DESIGN AND METHODS

Subjects in the present study are part of a longitudinal study of the etiology of type 2 diabetes among the Gila River Indian Community in Arizona, where most of the residents are Pima Indians or of the closely related Tohono O'odham tribe. Diabetes status was determined by an oral glucose tolerance test according to the criteria of the World Health Organization (10). The initial genetic study was conducted in a population-based sample of full-heritage Pima Indians (n = 3,501), where 1,561 subjects had type 2 diabetes (37% male, age at the last exam 49 ± 14 years, and BMI 39 ± 8 kg/m^2) and 1,940 subjects were nondiabetic (46% male, age at the last exam 31 ± 15 years, and BMI 36 ± 8 kg/m^2). Independent replication was assessed in 3,723 subjects from the same longitudinal study who were of mixed Native American heritage (reported heritage, on average, was one-half Pima and three-quarters Native American). The replication sample had 750 subjects with type 2 diabetes (41% male, age at the last exam 42 ± 14 years, and BMI 38 ± 9 kg/m^2) and 2,973 nondiabetic subjects (47% male, age at the last exam 24 ± 11 years, and BMI 34 ± 8 kg/m^2). Additional replication was assessed in a case-control sample from the Old Order Amish (139 diabetic subjects and 347 subjects with normal glucose tolerance) as previously described (11).

Metabolic quantitative traits. Among the full-heritage Pima Indians, 415 subjects (58% male, age 27 ± 6 years, and BMI 34 ± 8 kg/m^2) had undergone detailed metabolic testing for risk factors that predict type 2 diabetes. These individuals were determined to be nondiabetic, and acute insulin response was only analyzed in subjects who had normal glucose tolerance. Glucose tolerance was determined by a 75-g oral glucose tolerance test (OGTT) with measurements of fasting and 30, 60, 120, and 180-min plasma glucose and

insulin concentrations (12). The acute insulin response to intravenous glucose was measured on a separate day from the OGTT. Blood samples were collected prior to a 25-g glucose intravenous bolus infusion and at 3, 4, 5, 6, 8, and 10 min after infusion. The acute insulin response was calculated as the mean increment in plasma insulin concentrations from 3–5 min (13). Insulin sensitivity was assessed using the hyperinsulinemic-euglycemic clamp technique as previously described (12,13). Body composition was estimated by underwater weighing until January 1996 and by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation) thereafter (14).

SNP identification and genotyping. DNA from 24 Pima Indians (12 nondiabetic and aged >45 years; 12 diabetic with onset age <25 years) was sequenced using a Big Dye terminator (Applied Biosystems) on an automated DNA capillary sequencer (model 3730; Applied Biosystems). Genotyping was done using the SNPlex genotyping system 48-plex (Applied BioSystems) on an automated DNA capillary sequencer (model 3730; Applied Biosystems) for the Native American samples and Taqman genotyping assays (Applied Biosystems) for the Old Order Amish samples.

Statistical analysis. In the Pima study, statistical analyses were performed using the software of the SAS Institute (Cary, NC). The general association of genotypes with type 2 diabetes was assessed by logistic regression analysis and was adjusted for covariates (age, sex, and birth year). The model was fit with a generalized estimating equation technique to account for correlation among siblings. Genotype was analyzed as a numeric variable representing the number (0, 1, 2) of copies of a given allele. The association of quantitative traits with genotypes was analyzed by linear regression using the generalized estimating equation procedure to account for correlation among siblings. *P* values were adjusted for potential confounding covariates. In the replication study, which included individuals of mixed ancestry, the individual estimate of European admixture was also used as a covariate. These estimates were derived by the method of Hanis et al. (15) from 39 informative markers with large differences in allele frequency between populations (16). Linkage disequilibrium (LD) and haplotype blocks were estimated by Haploview (version 3.32).

For the Amish study, the odds ratio (OR) is derived from a logistic regression model, while the *P* value is based on the normal-liability threshold model implemented in SOLAR to account for relationships among individuals. CIs (and the approximate SE) are test based (17,18).

RESULTS AND DISCUSSION

Sequencing of the *MBL2* gene (all four exons, three introns, and ~2 kb of the upstream region) in 24 Pima Indians identified 37 variants. Three were previously known varients which predicted missense substitutions—rs5030737 (Arg52Cys), rs1800450 (Gly54Asp), and rs1800451 (Gly57Glu)—commonly referred to as A/D, A/B, and A/C, respectively (1–2). Three known promoter SNPs, rs11003125, rs7096206, and rs7095891, previously classified as −550 G > C (H/l), − 221G > C (Y/X), and +4 C > T (P/Q), respectively, were also identified (3). From these 37 SNPs in the *MBL2* gene, 12 tag SNPs were selected based on the tagger algorithm (Haploview 3.32, using $R^2 \geq 0.8$ to indicate redundancy [supplementary Fig. 1, available in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1593/DC1]). To additionally analyze variation flanking *MBL2* (~25 kb flanking each side of the sequenced region, chromosome 10:54170146–54228466), 20 database SNPs that serve as tag SNPs in the Chinese HapMap ($R^2 \geq 0.8$; minor allele frequency ≥0.1) were genotyped in Pima Indians, and it was determined that seven SNPs could serve as tag SNPs (defined above) in Pima samples (supplementary Fig. 1). All 19 tag SNPs (12 in *MBL2* and 7 flanking) were genotyped in the sample of 3,501 full-heritage Pima Indians for association analysis with type 2 diabetes (Table 1). The missense SNP rs1800450 (Gly54Asp, designated A/B) and the promoter SNP rs11003125 (designated H/l) were in high LD ($D' = 0.99$; $R^2 = 0.71$) and were associated with type 2 diabetes ($P = 0.002$ and 0.0007, respectively, adjusted for age, sex, birth year, and family membership; Table 1). These two tag SNPs captured several additional SNPs within intron 2 and the region near *MBL2*.

To determine whether the association with type 2 diabetes in the full-heritage Pima Indians could be replicated in other Native Americans, rs1800450 and rs11003125 were further genotyped in a nonoverlapping sample of 3,723 subjects who were predominately of mixed Native American heritage. The promoter SNP rs11003125 reproducibly associated with type 2 diabetes ($P = 0.04$, adjusted for age, sex, birth year, and heritage) (Table 1). Combining the initial and replication samples ($n = 7{,}224$) provided the strongest evidence for association with type 2 diabetes for rs11003125 ($P = 9.2 \times 10^{-5}$) (Table 1). The combined sample also showed a significant association for rs1800450 ($P = 0.001$) (Table 1), but this significant association was largely driven by the initial full-heritage Pima sample, with only a nonsignificant association in the same direction ($P = 0.18$) identified in the largely mixed-heritage replication group.

To determine whether variants in *MBL2* had a significant effect on diabetes in non–Native American populations, rs1800450 and rs11003125 were genotyped in an Amish sample of 139 diabetic subjects and 347 subjects with normal glucose tolerance. Consistent with the Native American samples, rs11003125 was associated with type 2 diabetes in the Amish (OR 1.51; $P = 0.004$, adjusted for age, sex, and family structure) (Table 2), but the frequency of risk allele G was lower in the Amish than in Pima Indians (0.45 vs. 0.77, respectively). The SNP rs1800450 (Gly54Asp) was also associated with type 2 diabetes in the Amish (OR 2.38, adjusted $P = 0.003$) (Table 2), but the frequency of risk allele glycine was comparable in the Amish and Pima Indians (0.87 vs. 0.83). In contrast, these associations do not appear to replicate in the large Caucasian Diabetes Genetics Replication and Meta-analysis (DIAGRAM) (19). Neither of these SNPs were directly genotyped in genome-wide association (GWA) studies from the Diabetes Genetics Initiative (DGI) and the Wellcome Trust Case Control Consortium (WTCCC), which are two of the three large studies from which the meta-analysis was derived; however, a proxy, rs1838065, which we determined to have an $R^2 = 1$ with rs11003125 (based on our genotyping of 90 Caucasians), was not associated with type 2 diabetes in DIAGRAM ($P = 0.35$, Table 2). Combining the negative DIAGRAM data together with the positive Amish and Native American data rendered the overall combined association nonsignificant ($P = 0.17$) (Table 2).

Data across a larger genomic region encompassing *MBL2* could also be obtained from prior GWA studies in Pima Indians (20), DGI (21), WTCCC (22), and DIAGRAM (19). For example, a ~400 kb region encompassing the ~8.3 kb *MBL2* (chromosome 10: 54007027–54401287) yielded 101 SNPs that were previously genotyped in a GWA study of Pima Indians. Only one GWA study SNP (rs1838065) was located within *MBL2* (intron 2). The pairwise LD pattern of these 101 SNPs and their association with early-onset type 2 diabetes in Pima subjects who were analyzed in this prior GWA (300 early-onset diabetes case and 334 control subjects) are shown in supplementary Fig. 2*A* and *B*. GWA study SNP rs1838065, which is in near-perfect LD with the promoter rs11003125 ($R^2 = 0.99$), was associated with early-onset type 2 diabetes (defined as diabetes onset ≤25 years of age) in the GWA study (adjusted $P = 0.0006$), as were several nearby GWA study SNPs (adjusted $P = 0.0007$–0.005) (supplementary Fig. 2), which were in high LD among themselves ($R^2 = 0.66$–0.99) but in low LD with rs1838065 ($R^2 = 0.30$–0.37). In

TABLE 1
Associations of *MBL2* tag SNPs with type 2 diabetes in Pima Indians

SNP	Location	Risk/ non-risk	Full-heritage Pima Indian (n = 3,501) AF	OR (95% CI)	$P_{additive}$	Replication mixed heritage (n = 3,723) AF	OR (95% CI)	$P_{additive}$	Combined (n = 7,224) OR (95% CI)	$P_{additive}$
rs920727	3′-flanking	T/C	0.90	1.23 (1.01–1.51)	0.05					
rs11003107	3′-flanking	C/T	0.99	1.04 (0.69–1.56)	0.85					
rs11003120	3′-flanking	C/T	0.99	4.05 (0.87–18.5)	0.07					
rs10082466	3′UTR	T/C	0.92	1.12 (0.89–1.40)	0.32					
Novel: 3′UTR	3′UTR	A/G	0.08	1.12 (0.90–1.42)	0.32					
rs930507	Leu126Leu	C/G	0.98	1.27 (0.86–1.88)	0.22					
rs10824793	Intron 2	A/G	0.95	1.27 (0.97–1.65)	0.08					
rs55902142	Intron 2	A/G	0.01	1.76 (1.00–3.10)	0.05					
rs1800451	Gly57Glu (A/C)	G/A	0.99	1.17 (0.14–9.80)	0.88					
rs1800450	Gly54Asp (A/B)	G/A	0.83	1.30 (1.10–1.53)	0.002	0.83	1.12 (0.94–1.40)	0.18	1.24 (1.09–1.40)	0.001
rs5030737	Arg52Cys (A/D)	C/T	0.99	1.95 (0.47–8.06)	0.35					
rs7095891	Promoter (P/Q)	C/T*	0.97	1.28 (0.88–1.88)	0.19	0.91	1.05 (0.80–1.39)	0.69	1.13 (0.91–1.41)	0.28
rs7096206	Promoter (Y/X)	G/C*	0.99	2.20 (0.92–5.21)	0.07					
rs11003125	Promoter (H/l)	G/C*	0.77	1.30 (1.12–1.51)	0.0007	0.68	1.19 (1.01–1.41)	0.04	1.25 (1.12–1.40)	9.2×10^{-5}
Novel: promoter	Promoter	G/A	0.98	1.20 (0.69–2.08)	0.51					
rs11003132	5′-flanking	C/T	0.99	1.47 (0.79–2.74)	0.22					
rs10762885	5′-flanking	A/G	0.97	1.24 (0.87–1.76)	0.22					
rs11003138	5′-flanking	C/T	0.91	1.14 (0.92–1.40)	0.23					
rs10824804	5′-flanking	A/C	0.88	1.28 (1.05–1.57)	0.02					

Nineteen tag SNPs ($R^2 \geq 0.8$) were selected from 57 SNPs that span *MBL2* (~8.3 kb) and approximately 25 kb flanking each side of the gene. Allele frequency (AF) is presented as frequency of the risk allele. OR is expressed as per copy of the risk allele (and thus, by definition, is >1). The risk allele is underlined where the P value is ≤0.05. *Genotypes were determined according to the reverse strand of the SNP database sequence. Sequences flanking the two novel SNPs are as follows: novel: promoter, tttcatggatgggtgtgtgc[g/a]tgcatgcacgtgtctgtgtg; novel: 3′UTR, catgactgcacagtaatttc[g/a]tctgtttataaacattgtat.

contrast with rs1838065, these additional GWA study SNPs (tagged by rs920727) had only a borderline association with type 2 diabetes (defined as diabetes at any age) in the current larger study of 3,501 full-heritage Pima subjects (rs920727, $P = 0.05$) (Table 1). GWA study data across this region in the DGI and WTCCC studies and DIAGRAM are shown in supplementary Fig. 3. Overall, there were no consistent associations with type 2 diabetes across the region in any of these Caucasian studies. When GWA study results of Pimas were compared with other populations in the *MBL2* region, rs1838065 was not significant in DIAGRAM. SNPs tagged by rs17587392 in Caucasians had a borderline significance with type 2 diabetes in DIAGRAM ($P = 0.05$) (supplementary Fig. 3) but was nearly monomorphic (minor allele frequency <0.001) and therefore uninformative in full-heritage Pima Indians, while SNP rs11003132, which had a borderline association with type 2 diabetes in WTCCC ($P = 0.05$) (supplementary Fig. 3), was not associated with type 2 diabetes in Pima Indians (Table 1).

TABLE 2
Association of promoter rs1103125 and rs1800450 (Gly54Asp) with type 2 diabetes in Pima Indian, Amish, and DIAGRAM subjects

	rs11003125 OR (95% CI)	P	P_{het}	rs1800450 OR (95% CI)	P	P_{het}
Native American						
Full-heritage Pima Indian	1.30 (1.12–1.51)	0.0007		1.30 (1.10–1.53)	0.002	
Replication mixed heritage	1.19 (1.01–1.41)	0.04		1.15 (0.94–1.40)	0.18	
Combined	1.25 (1.12–1.40)	9.2×10^{-5}	0.45	1.24 (1.09–1.40)	0.001	0.35
Caucasian						
Amish	1.51 (1.10–2.08)	0.004		2.38 (1.36–4.17)	0.003	
DIAGRAM*	0.97 (0.91–1.03)	0.35				
Combined	0.99 (0.93–1.05)	0.66	0.008			
Native American and Caucasian combined	1.04 (0.98–1.10)	0.17	0.0002			

A combined test was conducted by the inverse variance method. ORs are per copy of the risk allele identified in the full-heritage Pima sample. P is for the null hypothesis that the OR = 1. P_{het} is the P value for the null hypothesis that the ORs are the same for the combined groups. *DIAGRAM results based on rs1838065, which has an R^2 of 1.0 with rs1103125.

TABLE 3
Metabolic characteristics of full-heritage nondiabetic subjects grouped by genotypes of promoter rs11003125 or rs1800450 (Gly54Asp) variants

	rs11003125 (G/C)				rs1800450 (G/A)			
	G/G	G/C	C/C	$P_{additive}$	G/G (Gly/Gly)	G/A (Gly/Asp)	A/A (Asp/Asp)	$P_{additive}$
Nondiabetic subjects								
n	233	134	16		271	108	16	
Percent body fat	32 ± 0.5	33 ± 0.7	34 ± 1.9	0.60	32 ± 0.5	33 ± 0.8	36 ± 1.8	0.86
BMI (kg/m^2)	33 ± 0.5	33 ± 0.6	34 ± 2.2	0.56	33 ± 0.4	33 ± 0.7	37 ± 2.0	0.21
Fasting plasma glucose (mg/dl)	90 ± 0.6	89 ± 0.8	87 ± 2.2	0.09	89 ± 0.5	89 ± 0.8	89 ± 2.5	0.29
60-min plasma glucose (mg/dl)	150 ± 2.1	146 ± 3.0	137 ± 7.3	0.02	149 ± 2.0	145 ± 3.5	146 ± 7.7	0.12
2-h plasma glucose (mg/dl)	125 ± 1.9	122 ± 2.6	106 ± 6.1	0.0005	124 ± 1.8	122 ± 2.9	116 ± 8.0	0.04
Log fasting plasma insulin (μU/ml)	1.55 ± 0.01	1.54 ± 0.01	1.55 ± 0.05	0.31	1.56 ± 0.01	1.52 ± 0.01	1.62 ± 0.04	0.17
Log 2-h plasma insulin (μU/ml)	2.22 ± 0.02	2.15 ± 0.02	2.09 ± 0.07	0.0008	2.21 ± 0.02	2.13 ± 0.03	2.17 ± 0.07	0.003
Log glucose disposal rate under low-dose insulin clamp (mg · kg EMBS^{-1} · min^{-1})	0.54 ± 0.01	0.55 ± 0.01	0.53 ± 0.02	0.34	0.54 ± 0.01	0.56 ± 0.01	0.50 ± 0.02	0.32
Normal glucose tolerant subjects								
n	164	99	9		192	80	9	
Log acute insulin response (μU/ml)	2.33 ± 0.02	2.35 ± 0.02	2.49 ± 0.07	0.14	2.32 ± 0.04	2.37 ± 0.02	2.52 ± 0.02	0.004
Log 30-min plasma insulin (μU/ml)	2.34 ± 0.01	2.34 ± 0.02	2.41 ± 0.13	0.31	2.34 ± 0.01	2.35 ± 0.02	2.40 ± 0.12	0.27

Data are means ± SE unless otherwise indicated. Plasma insulin concentrations (fasting, 2 h, and 30 min), rates of glucose disappearance during the low-dose insulin stimulation, and the acute insulin response were log transformed before analyses to approximate a normal distribution. The *P* value for percent body fat was adjusted for age, sex, and family membership. The *P* value for the acute insulin response was adjusted for age, sex, percent body fat, and rate of glucose disappearance during the low-dose insulin stimulation. All remaining *P* values (except for age) were adjusted for age, sex, percent body fat, and family membership. EMBS, estimated metabolic body size.

The observation that specific *MBL2* SNPs had replicated associations with type 2 diabetes in Native Americans and a small group of Amish subjects, who are of European descent but were not associated with diabetes in the large DIAGRAM, is unexpected. It is possible that the association in the Amish is a false positive. Alternatively, both the Pima Indians and the Old Order Amish represent far more homogeneous populations compared with the study population of DIAGRAM. It is also possible that susceptibility genes for common diseases may have larger effects in these populations compared with others as a result of segregation of high-penetrance alleles that are rare or nonexistent in the general population, gene-gene or gene-environment interactions, or the absence of other susceptibility genes whose effects could mask other genes.

To aid in validating the positive associations with type 2 diabetes, we further investigated whether these variants in *MBL2* were associated with metabolic risk factors that predict type 2 diabetes among 415 nondiabetic, full-heritage Pima Indians. For both rs11003125 and rs1800450, the allele associated with higher risk for diabetes (G and glycine, respectively) was associated with a higher 2-h plasma glucose concentration (adjusted $P = 0.0005$ and 0.04, respectively) and higher 2-h plasma insulin concentration (adjusted $P = 0.0008$ and 0.003, respectively) during an oral glucose tolerance test (Table 3). The risk allele glycine for rs1800450 was additionally associated with a lower acute insulin response to an intravenous glucose bolus infusion (adjusted $P = 0.004$) among subjects who had normal glucose tolerance ($n = 281$) (Table 3). However, neither SNP was associated with insulin-stimulated glucose uptake.

Previous functional studies have shown that serum MBL levels are greatly influenced by variants within the *MBL2* gene. Three promoter variants (rs11003125 [H/l], rs7096206 [Y/X], and rs7095891 [P/Q]) and three missense variants (rs5030737 [A/D], rs1800450 [A/B], and rs1800451 [A/C]) were previously associated with MBL deficiency (3,23). Several of these variants are in high LD, so a limited number of haplotypes are present in humans. *HYPA* (*G*-G-C-*Gly*) haplotype carriers have the highest serum concentration of MBL, typically from 1,400 to 2,500 μg/l, whereas *LYPB* (*C*-G-C-*Asp*) haplotype carriers have the lowest serum concentration: 20–400 μg/l (3,20). Since rs7096206 (Y/X) and rs7095891 (P/Q) are very rare in Pima Indians (minor allele frequency <0.03), these high versus low serum level haplotypes can essentially be determined from a two-SNP haplotype of the promoter rs11003125 (H/l) and missense rs1800450 (A/B) in Pima Indians.

Haplotype analysis for rs11003125 and rs1800450 was performed in the combined sample of 7,224 predominately Pima Indians. The haplotype G-glycine (HA), carrying both the G nucleotide (designated H allele) of the promoter rs11003125 and glycine (designated A allele) of rs1800450, was associated with increased risk for type 2 diabetes in Pima Indians (haplotype frequency of G-glycine 0.78 in diabetic vs. 0.71 in nondiabetic; OR 1.25 [95% CI 1.12–1.33], $P = 0.0001$, adjusted for age, sex, and birth year [heritage estimate in the replication group]). Because this haplotype G-glycine is highly concordant with the G (H) allele of rs11003125 ($R^2 = 0.99$), it is difficult to statistically distinguish the single genotypic versus haplotypic effects. When both SNPs are included in a single model, there is a significant association with rs11003125 (OR 1.29 [95% CI 1.08–1.54]; $P = 0.005$) conditional on the effect at rs1800450, but there is little association with rs1800450

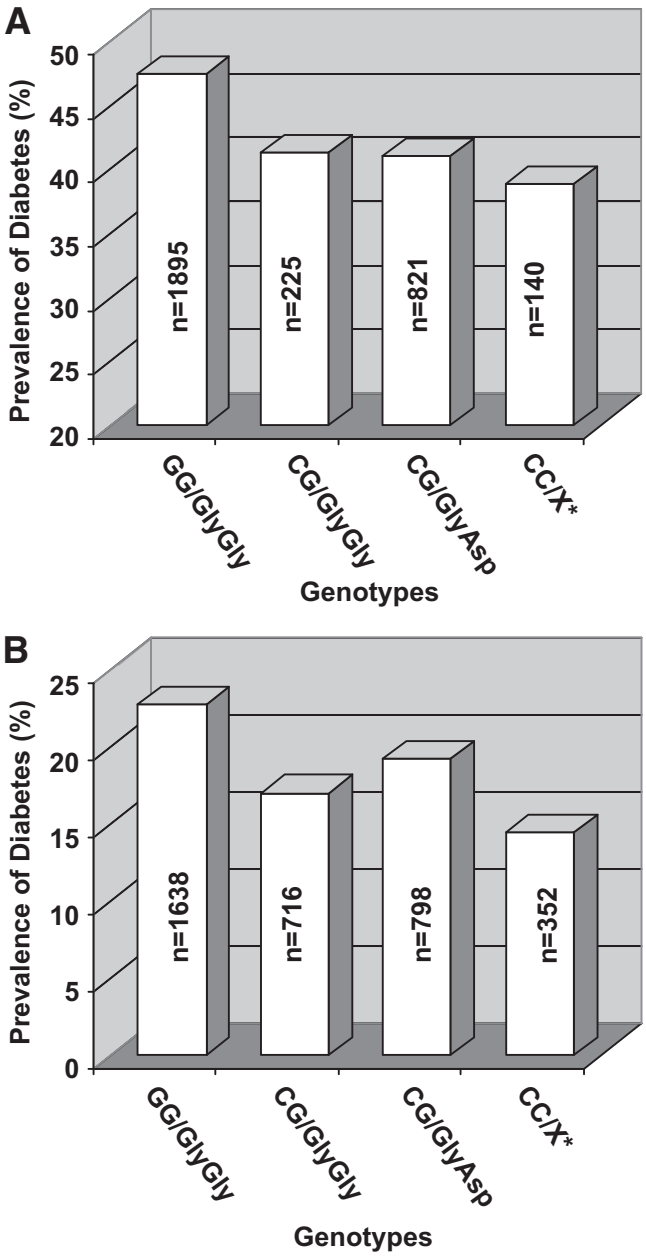

FIG. 1. Prevalence of type 2 diabetes in the full-heritage Pima Indian group (*A*) (n = 3,081) and the replication mixed heritage group (*B*) (n = 3,504) according to genotypes of promoter rs11003125 and rs1800450 (Gly54Asp). *Either homozygous or heterozygous for the Asp alleles.

conditional on the effect at rs1103125 (0.94 [0.77–1.16]; P = 0.61). It thus appears that the promoter rs11003125 is the stronger predictor of diabetes, with little additional information from rs1800450.

The prevalence of type 2 diabetes was plotted in the full-heritage Pima Indian (n = 3,081) (Fig. 1*A*) and the replication Native American (n = 3,504) (Fig. 1*B*) groups according to the genotypes of rs11003125 and rs1800450. Ordering the genotypic groups according to their association with serum MBL levels showed that subjects homozygous for both G and glycine alleles had a higher prevalence of diabetes than did subjects homozygous for the C allele and either homozygous or heterozygous for the aspartic acid alleles ($P_{trend} = 8.4 \times 10^{-5}$ in the combined analysis of 6,585 predominately Pima subjects, adjusted for age, sex, and birth year, and in the replication group, heritage). In Caucasians, Eskimos, Africans, South Americans, and Native Americans, the G-glycine (HA) haplotype is associated with higher MBL serum level (3,24–26).

The present study demonstrates that an allele for *MBL2*, which arises predominately from the promoter SNP rs11003125 (G allele), predicts a higher serum level of MBL2 and is associated with increased risk for type 2 diabetes, increased 2-h plasma glucose and 2-h plasma insulin, and decreased insulin secretion in some populations. Consistent with our observations, high MBL2 levels have recently been reported to be associated with high A1C levels in the Strong Heart Study, a longitudinal study of cardiovascular disease among Native Americans (26). SNP rs11003125 is in high LD with other SNPs that map within intron 2 and a flanking region of *MBL2*; therefore, the contribution of other functional SNPs cannot be ruled out. Nevertheless, both high LD and diabetes association were restricted to the region in and near *MBL2*, suggesting that evidence for association with type 2 diabetes is more likely derived from *MBL2* rather than other genes in the region.

Although the physiologic mechanisms underlying the association of MBL2 levels with diabetes are unknown, it has previously been shown that MBL2 plays a dual role in modifying inflammatory responses (27). Deficiency of MBL has been linked to increased risk of developing type 1 diabetes (6), insulin resistance, and obesity (9) as a result of a chronic infectious state or low-grade inflammation. MBL2 could also affect metabolic pathways through stimulating fatty acid oxidation in skeletal muscle (28) or reducing release of tumor necrosis factor-α, interleukin-1, and interleukin-6 (29). In contrast, increased MBL levels could lead to an overly activated complement system, thereby inducing inflammatory damage or interweaving a complex autoimmune process (30). Consistent with the latter effect, high MBL levels have been associated with increased risk for insulin resistance in pregnancy (31) and late-onset of rheumatoid arthritis (5). However, our study indicates that *MBL2* variants are more likely to influence type 2 diabetes via an effect on insulin secretion rather than on insulin action, suggesting that inflammatory damage in pancreatic β-cell function may be involved. Additional studies are needed to investigate the impact of this gene on specific type 2 diabetes related–pathways and disease susceptibility in non–Native American groups.

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Yunhua L. Muller researched data, wrote manuscript. Robert L. Hanson reviewed/edited manuscript, contributed to discussion. Li Bian researched data, contributed to discussion. Janel Mack researched data. Xiaolian Shi researched data. Ruth Pakyz researched data. Alan R. Shuldiner reviewed/edited manuscript, contributed to discussion. William

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