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Original Contribution

Diabetes and Obesity-Related Genes and the Risk of Neural Tube Defects in the National Birth Defects Prevention Study

Philip J. Lupo, Mark A. Canfield, Claudia Chapa, Wei Lu, A. J. Agopian, Laura E. Mitchell, Gary M. Shaw, D. Kim Waller, Andrew F. Olshan, Richard H. Finnell, and Huiping Zhu*

* Correspondence to Dr. Huiping Zhu, Dell Pediatric Research Institute, Department of Nutritional Sciences, University of Texas at Austin, Campus Mail Code R1800, 1400 Barbara Jordan Blvd., Austin, TX 78723 (e-mail: hzhu@austin.utexas.edu).

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Few studies have evaluated genetic susceptibility related to diabetes and obesity as a risk factor for neural tube defects (NTDs). The authors investigated 23 single nucleotide polymorphisms among 9 genes (*ADRB3*, *ENPP1*, *FTO*, *LEP*, *PPARG*, *PPARGC1A*, *SLC2A2*, *TCF7L2*, and *UCP2*) associated with type 2 diabetes or obesity. Samples were obtained from 737 NTD case-parent triads included in the National Birth Defects Prevention Study during 1999–2007. Log-linear models were used to evaluate maternal and offspring genetic effects. After application of the false discovery rate, there were 5 significant maternal genetic effects. The less common alleles at the 4 *FTO* single nucleotide polymorphisms showed a reduction of NTD risk (for rs1421085, relative risk (RR) = 0.73 (95% confidence interval (CI): 0.62, 0.87); for rs8050136, RR = 0.79 (95% CI: 0.67, 0.93); for rs9939609, RR = 0.79 (95% CI: 0.67, 0.94); and for rs17187449, RR = 0.80 (95% CI: 0.68, 0.95)). Additionally, maternal *LEP* rs2071045 (RR = 1.31, 95% CI: 1.08, 1.60) and offspring *UCP2* rs660339 (RR = 1.32, 95% CI: 1.06, 1.64) were associated with NTD risk. Furthermore, the maternal genotype for *TCF7L2* rs3814573 suggested an increased NTD risk among obese women. These findings indicate that maternal genetic variants associated with glucose homeostasis may modify the risk of having an NTD-affected pregnancy.

case-parent triads; diabetes; genetics; neural tube defects; obesity

Abbreviations: CI, confidence interval; NBDPS, National Birth Defects Prevention Study; NTDs, neural tube defects; RR, relative risk; SNP, single nucleotide polymorphism.

Neural tube defects (NTDs) are among the most common, most costly, and most deadly of all human congenital anomalies whose etiologies remain largely unknown (1, 2). NTDs include a range of malformations (e.g., spina bifida, anencephaly), which further complicates the identification of risk factors. Two well-established risk factors for NTDs are maternal pregestational diabetes and prepregnancy obesity (3–12). Although mechanisms underlying these risks remain unclear, there is evidence that infants born to obese mothers and infants born to diabetic mothers may share some common underlying pathogenic exposures, including alteration of glucose homeostasis and hyperglycemia (13–18).

Glucose is monitored and regulated by the pancreas and is an essential fuel for oxidative metabolism. During early organogenesis, there is high demand for glucose, since the embryo is dependent on uninterrupted anaerobic glycolysis before the chorioallantoic placenta is developed. Evidence suggests that the early embryo does not have pancreatic function until the development of β cells, which occurs after the seventh week of gestation (19). Thus, at the time of neural tube closure (approximately the fourth week of gestation), mothers with poorly regulated glucose levels are likely to have an altered in utero environment, which cannot be managed by the developing embryo, leading to abnormal organogenesis (20–22).

Several genes related to glucose homeostasis have been previously identified in human and animal studies. Furthermore, genes related to glucose homeostasis have been associated with type 2 diabetes and obesity risk in genome-wide

association studies (23, 24). A few studies have investigated some of these genes in relation to NTD risk, with positive findings (25, 26); however, these analyses have been limited to a small number of single nucleotide polymorphisms (SNPs) or have not assessed the role of maternal genetic effects. Therefore, our objective in this study was to investigate the roles of several maternal and offspring genes related to glucose homeostasis in the risk of NTDs.

MATERIALS AND METHODS

Study population

The study population included NTD case-parent triads ($n = 737$) from the National Birth Defects Prevention Study (NBDPS), with estimated dates of delivery between January 1, 1999, and December 31, 2007. Details on the NBDPS have been published elsewhere (27). In brief, the NBDPS is a population-based case-control study of major structural birth defects. For the period 1999–2007, case infants with one or more congenital anomalies were ascertained through 10 birth defects surveillance systems throughout the United States (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah) and included livebirths, stillbirths, and induced abortions (pregnancy terminations). NTDs included in the NBDPS had British Pediatric Association codes for the diagnoses anencephaly (740.0), craniorachischisis (740.1), spina bifida (741.0), and encephalocele (742.0). Abstracted data for all NTD case infants were reviewed by clinical geneticists using specific criteria, including standardized case definitions and confirmatory diagnostic procedures (28). Infants/fetuses with known single gene disorders or chromosomal abnormalities were excluded from the NBDPS. Mothers completed a 1-hour computer-assisted telephone interview in English or Spanish between 6 weeks and 2 years after the estimated date of delivery. The interview included sections on maternal conditions and illnesses, lifestyle and behavioral factors, and multivitamin use.

Candidate genes and SNPs

Candidate genes and SNPs were selected if 1) they were identified as being associated with type 2 diabetes or obesity in multiple genome-wide association studies (i.e., *TCF7L2* and *FTO*) (23, 29) or 2) there was evidence from candidate gene studies coupled with biologic plausibility supported by studies using animal models (e.g., *ADRB3*, *ENPP1*, *UCP2*, *LEP*, *SLC2A2*, *PPARG*, and *PPARGC1A*) (30–35). The selection criteria for each candidate gene and SNP are presented in Web Table 1, which appears on the *Journal*'s website (http://aje.oxfordjournals.org/).

DNA samples and genotyping analysis

Buccal swabs were collected from mothers, fathers, and infants as part of the NBDPS (36). DNA was extracted from buccal cells. A standard quality control procedure was applied to each sample before it was submitted to the NBDPS sample repository (36). To assure genotyping proficiency, high quality, and high concordance among all NBDPS laboratories, annual evaluations are conducted to confirm the performance of each laboratory (see Web Appendix). Our laboratory at the Dell Pediatric Research Institute (University of Texas at Austin) has passed all of these evaluations with a score of 100%. SNPs were assayed using TaqMan (Life Technologies Corporation, Carlsbad, California), and genotypes were read and distinguished on

Table 1. Characteristics of Neural Tube Defect Case-Parent Triads ($n = 737$), National Birth Defects Prevention Study, 1999–2007

Characteristic	No. of Triads	%
Phenotype		
Spina bifida	449	60.9
Anencephaly	217	29.4
Encephalocele	71	9.6
Infant sex		
Male	337	47.9
Female	366	52.1
Maternal age, years		
<20	83	11.3
20–34	556	75.4
≥35	98	13.3
Race/ethnicity		
Non-Hispanic white	439	59.8
Non-Hispanic black	34	4.6
Hispanic	221	30.1
Other	40	5.5
Education, years		
<12	142	19.3
12	184	25.0
13–15	226	30.7
>15	185	25.0
Folic acid supplementation[a]		
No	351	47.6
Yes	386	52.4
Body mass index[b] category		
Underweight (<18.5)	28	4.1
Normal weight (18.5–24.9)	336	48.6
Overweight (25.0–29.9)	152	21.9
Obese (≥30)	176	25.4
Prepregnancy diabetes		
No	724	98.2
Yes	13	1.8
Gestational diabetes		
No	667	95.8
Yes	29	4.2

[a] Use of folic acid supplements from 3 months before conception through the first month of pregnancy.
[b] Weight (kg)/height $(m)^2$.

the ABI PRISM 7900HT Sequence Detection System (Life Technologies Corporation).

Statistical analysis

The characteristics of case subjects and their parents were summarized using counts and proportions. For each analyzed variant, samples for which a genotype could not be assigned and triads with genotype combinations incompatible with Mendelian inheritance were determined. For each sample, the number of genotyping failures (i.e., genotypes that could not be assigned) was determined. These analyses were performed using Intercooled Stata, version 10.1 (StataCorp LP, College Station, Texas).

Log-linear models were used to assess the association between NTDs and both the offspring and maternal genotypes for each variant (37, 38). To more fully adjust for the effect not being directly assessed, a log-additive model of inheritance was assumed for the genotype being assessed (e.g., the maternal genotype) and an unrestricted model of inheritance was used for the other genotype (e.g., the offspring genotype). This approach provides a 1-degree-of-freedom test for the effect under study. Genotype relative risks and 95% confidence intervals were estimated. In addition, *P* values for offspring and maternal genetic effects were determined using likelihood ratio tests to compare the log-linear model including terms for both the offspring and maternal genotypes with reduced models that included terms for only the offspring genotype or only the maternal genotype. These analyses were carried out using the LEM program, which allows for the inclusion of incompletely genotyped triads (39, 40).

Analyses were conducted using data from all complete and incomplete triads (i.e., the full group) and stratified according to maternal prepregnancy obesity status (i.e., body mass index (weight (kg)/height $(m)^2$) $\geq$30 vs. <30), as several of these variants have been associated with obesity. We did not formally assess statistical interactions because of sample size considerations. Additionally, analyses were conducted in 3 subgroups: 1) triads with spina bifida only; 2) triads in which mothers did not have pregestational diabetes; and 3) triads in which mothers did not have pregestational or gestational diabetes. These subgroups were assessed to determine whether the results obtained using data from all triads were influenced by heterogeneity within the full group. We did not stratify on maternal pregestational and gestational diabetes due to small numbers. Because of concerns about population stratification bias when assessing maternal genetic effects, the analyses of the full NTD group were repeated among non-Hispanic whites (37). Finally, due to the number of comparisons, the Benjamini and Hochberg method (the false discovery rate) was used to calculate a "corrected" *P* value (*Q* value) accounting for multiple tests in the full group (41).

RESULTS

Participation in the NBDPS for the period 1999–2007 was 74% among NTD case mothers, yielding 1,553 families available for analysis. Among those, 759 (49%) provided buccal swabs (1,787 individuals). Genotyping was performed on DNA samples derived from these 759 families. Based on quality control checks, 18 families (2% of families) were excluded for being inconsistent with Mendelian inheritance at more than 2 genotypes. Additionally, 47 subjects were excluded for failure at more than 11 genotypes (>50%), resulting in 4 more triads' being excluded and leaving a total of 737 case-parent triads (97% of the original sample). Of those, 317 were complete triads, 313 were dyads, and 107 were monads with only 1 person in the family. After these quality control measures were applied, at least 95% of the samples for each variant were available for analyses; therefore, the genotypes were considered of sufficiently high quality.

The distributions of key characteristics among NTD case-parent triads are presented in Table 1. Spina bifida was the most common phenotype among case subjects ($n = 449$; 60.9%). Furthermore, a majority of case mothers were non-Hispanic white ($n = 439$; 59.8%). Among case mothers, 176 were obese (25.4%), 13 had prepregnancy diabetes (1.8%), and 29 had gestational diabetes (4.2%). The only characteristics presented in Table 1 that were significantly different between interviewed case mothers who provided buccal swabs and those who did not were race/ethnicity and education (data not shown).

Table 2 shows estimated relative risks (heterozygote vs. common homozygote) and 95% confidence intervals for the association between offspring and maternal genotypes and NTDs, as well as the likelihood ratio test *P* values and false discovery rate *Q* values for the model comparisons for each variant. Offspring genotypes for *ADRB3*, *ENPP1*, *FTO*, *LEP*, *PPARG*, *PPARGC1A*, *SLC2A2*, or *TCF7L2* were not associated with NTD risk. However, the offspring genotype for *UCP2* rs660339 was associated with NTD risk (relative risk (RR) = 1.32, 95% confidence interval (CI): 1.06, 1.64).

There was no statistical evidence of associations between maternal genotypes for *ADRB3*, *ENPP1*, *PPARG*, *PPARGC1A*, *SLC2A2*, or *UCP2* and the risk of NTDs in offspring (Table 2). However, the less common alleles of all *FTO* genotypes (rs1421085, rs8050136, rs9939609, and rs17817449) were negatively associated with NTD risk among mothers. In contrast, the less common alleles for *LEP* rs2071045 and *TCF7L2* rs3814573 were associated with an elevated risk among mothers (RR = 1.31 (95% CI: 1.08, 1.60) and RR = 1.22 (95% CI: 1.04, 1.44), respectively). Results were similar (e.g., the estimated relative risks were similar) when analyses were restricted to 1) spina bifida cases only, 2) mothers without pregestational diabetes, 3) mothers without pregestational or gestational diabetes, and 4) non-Hispanic whites; therefore, only results for the full group are presented.

When analyses were stratified on the basis of maternal body mass index (Tables 3 and 4), the effect of *TCF7L2* rs3814573 was stronger among obese women (RR = 1.64, 95% CI: 1.15, 2.33) than among nonobese women (RR = 1.11, 95% CI: 0.92, 1.35). Additionally, none of the *FTO* genotypes were significantly associated with NTD risk in obese women, whereas these variants were associated with NTD risk in nonobese women. Offspring genetic

Table 2. Log-Linear Results for the Association Between Diabetes and Obesity-Related Genes and the Risk of Neural Tube Defects, National Birth Defects Prevention Study, 1999–2007

Variant	No. of Triads	No. of Dyads	No. of Monads	Offspring Genetic Effect				Maternal Genetic Effect			
				RR[a]	95% CI	LRT *P* Value	LRT *Q* Value	RR[a]	95% CI	LRT *P* Value	LRT *Q* Value
ADRB3 rs4994	312	316	108	1.16	0.86, 1.57	0.33	0.71	0.88	0.68, 1.14	0.34	0.78
ENPP1 rs1044498	312	304	120	0.93	0.70, 1.24	0.62	0.79	1.11	0.91, 1.35	0.30	0.76
FTO											
rs1421085	278	309	126	0.86	0.68, 1.10	0.24	0.69	0.73	0.62, 0.87	0.0003	0.007
rs8050136	300	317	112	0.84	0.67, 1.05	0.13	0.55	0.79	0.67, 0.93	0.0048	0.03
rs9939609	302	298	125	0.81	0.64, 1.01	0.06	0.55	0.79	0.67, 0.94	0.0054	0.03
rs17817449	292	324	116	0.82	0.66, 1.03	0.09	0.55	0.80	0.68, 0.95	0.0092	0.04
LEP											
rs2071045	295	315	116	1.25	0.96, 1.63	0.10	0.55	1.31	1.08, 1.60	0.0064	0.03
rs2167270	299	317	120	0.95	0.77, 1.17	0.64	0.79	0.99	0.85, 1.17	0.94	0.99
rs3828942	303	315	119	1.07	0.87, 1.33	0.52	0.79	0.97	0.83, 1.13	0.70	0.99
rs11760956	296	311	121	0.94	0.76, 1.17	0.59	0.79	0.95	0.81, 1.11	0.52	0.99
rs12706831	307	314	112	1.12	0.91, 1.38	0.30	0.71	1.00	0.85, 1.17	0.98	0.99
PPARG rs1801282	311	309	113	1.05	0.74, 1.49	0.78	0.85	1.04	0.80, 1.34	0.79	0.99
PPARGC1A											
rs3736265	293	314	110	1.01	0.64, 1.60	0.97	0.97	1.18	0.87, 1.60	0.30	0.76
rs8192678	302	313	111	1.02	0.81, 1.28	0.88	0.92	1.01	0.86, 1.19	0.87	0.99
SLC2A2											
rs5400	303	316	114	1.23	0.91, 1.67	0.17	0.55	1.00	0.80, 1.24	0.99	0.99
rs6785233	311	309	113	1.19	0.83, 1.72	0.34	0.71	0.92	0.70, 1.20	0.53	0.99
rs11924032	306	308	119	0.84	0.66, 1.07	0.16	0.55	1.00	0.84, 1.18	0.99	0.99
TCF7L2											
rs290487	317	312	107	1.12	0.86, 1.47	0.40	0.76	1.03	0.84, 1.26	0.77	0.99
rs3814573	309	312	113	1.03	0.84, 1.28	0.75	0.85	1.22	1.04, 1.44	0.02	0.07
rs7903146	302	319	109	0.91	0.72, 1.15	0.43	0.76	0.96	0.80, 1.15	0.65	0.99
rs10885390	308	302	117	1.07	0.84, 1.38	0.58	0.79	0.99	0.83, 1.17	0.87	0.99
rs12255372	306	308	118	0.95	0.74, 1.21	0.66	0.79	0.88	0.73, 1.06	0.17	0.55
UCP2 rs660339	301	316	115	1.32	1.06, 1.64	0.01	0.23	0.97	0.83, 1.13	0.68	0.99

Abbreviations: CI, confidence interval; LRT, likelihood ratio test; RR, relative risk.
[a] Results are based on an additive model (i.e., the risk of being a heterozygote vs. the common homozygote).

effects also appeared to differ by maternal prepregnancy obesity (Tables 3 and 4). For instance, the association between NTD risk and *UCP2* rs660339 was stronger for the offspring of obese women (RR = 1.74, 95% CI: 1.14, 2.64) than for the offspring of nonobese women (RR = 1.19, 95% CI: 0.91, 1.55). The offspring genetic effect of *LEP* rs3828942 also differed on the basis of maternal prepregnancy obesity, whereby the less common allele was associated with a reduced risk in offspring of obese women (RR = 0.66, 95% CI: 0.44, 0.98) and an increased risk in offspring of nonobese women (RR = 1.31, 95% CI: 1.00, 1.72). This was also true for *LEP* rs12706831, whereby the less common allele was associated with a reduced risk in offspring of obese women (RR = 0.69, 95% CI: 0.45, 1.06) and an increased risk in offspring of nonobese women (RR = 1.31, 95% CI: 1.01, 1.69).

Because of the number of comparisons, we applied the false discovery rate in the full group. Although none of the offspring genetic effects remained statistically significant, 5 of the 6 significant maternal genetic effects in Table 2 (the 4 *FTO* genotypes and *LEP* rs2071045) remained significant at $P < 0.05$.

DISCUSSION

We evaluated the risk of NTDs associated with maternal and offspring genetic effects of 23 SNPs for 9 diabetes and obesity-related genes. There were significant associations between maternal variants in *FTO*, *TCF7L2*, and *LEP*

Table 3. Log-Linear Results Among Obese Mothers for the Association Between Diabetes and Obesity-Related Genes and the Risk of Neural Tube Defects, National Birth Defects Prevention Study, 1999–2007

Variant	No. of Triads	No. of Dyads	No. of Monads	Offspring Genetic Effect			Maternal Genetic Effect		
				RR[a]	95% CI	LRT *P* Value	RR[a]	95% CI	LRT *P* Value
ADRB3 rs4994	91	63	23	1.29	0.73, 2.28	0.39	0.66	0.39, 1.14	0.13
ENPP1 rs1044498	90	62	25	0.84	0.49, 1.46	0.54	1.02	0.68, 1.52	0.94
FTO									
rs1421085	76	64	32	0.78	0.49, 1.25	0.30	0.77	0.54, 1.09	0.14
rs8050136	83	69	23	0.77	0.49, 1.20	0.24	0.95	0.70, 1.29	0.73
rs9939609	85	62	28	0.83	0.53, 1.29	0.41	0.93	0.69, 1.27	0.66
rs17817449	87	66	24	0.79	0.52, 1.21	0.28	1.01	0.73, 1.39	0.95
LEP									
rs2071045	78	68	29	1.19	0.65, 2.15	0.58	1.34	0.89, 2.03	0.15
rs2167270	89	59	29	1.48	0.98, 2.22	0.06	0.86	0.63, 1.17	0.34
rs3828942	85	69	23	0.66	0.44, 0.98	0.04	1.09	0.79, 1.52	0.60
rs11760956	85	64	25	1.49	0.98, 2.26	0.06	0.92	0.67, 1.25	0.59
rs12706831	85	67	23	0.69	0.45, 1.06	0.09	1.11	0.81, 1.51	0.52
PPARG rs1801282	88	64	23	0.91	0.48, 1.71	0.76	1.02	0.58, 1.78	0.95
PPARGC1									
rs3736265	80	69	24	0.72	0.27, 1.93	0.51	1.40	0.73, 2.69	0.31
rs8192678	86	65	22	0.87	0.57, 1.34	0.54	1.18	0.82, 1.69	0.37
SLC2A2									
rs5400	87	62	28	1.05	0.60, 1.83	0.86	1.03	0.65, 1.63	0.90
rs6785233	87	67	22	0.90	0.47, 1.74	0.75	0.78	0.43, 1.43	0.42
rs11924032	85	65	27	0.93	0.58, 1.48	0.76	0.86	0.59, 1.25	0.44
TCF7L2									
rs290487	90	63	24	1.15	0.68, 1.95	0.59	1.13	0.75, 1.71	0.55
rs3814573	88	65	24	1.21	0.78, 1.86	0.40	1.64	1.15, 2.33	0.0044
rs7903146	83	70	23	1.01	0.63, 1.62	0.97	1.15	0.81, 1.63	0.45
rs10885390	88	64	21	1.11	0.71, 1.75	0.65	0.99	0.69, 1.43	0.96
rs12255372	88	62	25	0.94	0.59, 1.51	0.81	0.98	0.68, 1.42	0.91
UCP2 rs660339	82	66	27	1.74	1.14, 2.64	0.01	1.07	0.77, 1.49	0.70

Abbreviations: CI, confidence interval; LRT, likelihood ratio test; RR, relative risk.
[a] Results are based on an additive model (i.e., the risk of being a heterozygote vs. the common homozygote).

genes and the risk of NTDs in offspring after applying the false discovery rate. Additionally, an offspring variant in the *UCP2* gene was associated with NTD risk, although this association did not remain significant after applying the false discovery rate.

The fat mass and obesity-associated gene (*FTO*) has been identified as a risk factor for obesity through several genome-wide association studies and has been confirmed in multiple populations (29, 42–44). Minor alleles of 4 common intronic SNPs—rs1421085 (C), rs8050136 (A), rs9939609 (A), and rs17817449 (G)—are associated with increased body mass, increased obesity risk, and increased FTO protein (α-ketoglutarate-dependent dioxygenase) expression (43, 45–49). The FTO protein is believed to play a role in controlling feeding behavior and energy expenditure. However, biologic mechanisms by which FTO contributes to common obesity remain unknown, partly because of discrepancies between animal studies and observations in humans (50–52). Minor alleles in the maternal genotypes of these 4 SNPs were significantly associated with a decreased NTD risk in offspring. Three variants (rs8050136, rs9939609, and rs17817449) are located in a 7-kilobase region in intron 1 of the *FTO* gene and are in strong linkage disequilibrium (47); however, rs1421085 is 12.5 kilobases away from this region and is not in linkage disequilibrium with the other variants. Because the minor alleles of these SNPs were negatively associated with NTD risk among nonobese mothers in our population, the *FTO* genotypes may be associated with NTDs through mechanisms other than maternal obesity (e.g., an ancestral survival advantage related to fat accumulation) (4, 9–12, 53).

The T-cell factor 7-like 2 gene (*TCF7L2*) harbors the variants with the strongest association with type 2 diabetes risk identified to date (23). In recent years, it has become

Table 4. Log-Linear Results Among Nonobese Mothers for the Association Between Diabetes and Obesity-Related Genes and the Risk of Neural Tube Defects, National Birth Defects Prevention Study, 1999–2007

Variant	No. of Triads	No. of Dyads	No. of Monads	Offspring Genetic Effect			Maternal Genetic Effect		
				RR[a]	95% CI	LRT *P* Value	RR[a]	95% CI	LRT *P* Value
ADRB3 rs4994	206	234	76	1.38	0.93, 2.03	0.10	0.94	0.69, 1.28	0.72
ENPP1 rs1044498	206	223	87	0.96	0.67, 1.37	0.83	1.13	0.89, 1.43	0.31
FTO									
rs1421085	184	230	85	0.91	0.68, 1.22	0.53	0.71	0.58, 0.88	0.0010
rs8050136	200	231	81	0.89	0.67, 1.17	0.39	0.74	0.60, 0.90	0.0030
rs9939609	201	218	88	0.83	0.63, 1.09	0.18	0.74	0.60, 0.90	0.0027
rs17817449	192	238	82	0.85	0.64, 1.12	0.24	0.74	0.60, 0.91	0.0032
LEP									
rs2071045	201	229	81	1.19	0.88, 1.63	0.26	1.33	1.06, 1.68	0.01
rs2167270	193	241	82	0.77	0.59, 1.00	0.05	1.05	0.87, 1.28	0.61
rs3828942	200	230	87	1.31	1.00, 1.72	0.05	0.92	0.77, 1.11	0.38
rs11760956	196	227	88	0.77	0.59, 1.01	0.05	0.96	0.79, 1.16	0.65
rs12706831	205	229	82	1.31	1.01, 1.69	0.04	0.96	0.79, 1.16	0.67
PPARG rs1801282	205	229	81	1.05	0.68, 1.62	0.84	1.03	0.76, 1.40	0.86
PPARGC1A									
rs3736265	193	232	77	1.01	0.59, 1.73	0.96	1.06	0.73, 1.54	0.75
rs8192678	198	231	81	1.11	0.84, 1.46	0.48	0.98	0.81, 1.18	0.83
SLC2A2									
rs5400	200	235	78	1.36	0.93, 1.98	0.11	1.08	0.83, 1.41	0.57
rs6785233	206	227	81	1.42	0.89, 2.27	0.14	1.01	0.74, 1.39	0.94
rs11924032	204	226	83	0.79	0.59, 1.06	0.11	1.06	0.87, 1.30	0.55
TCF7L2									
rs290487	209	232	75	1.16	0.84, 1.61	0.37	0.97	0.77, 1.23	0.82
rs3814573	203	231	80	0.94	0.72, 1.21	0.63	1.11	0.92, 1.35	0.27
rs7903146	200	234	77	0.84	0.64, 1.12	0.23	0.90	0.72, 1.13	0.36
rs10885390	203	220	88	1.07	0.78, 1.46	0.66	0.98	0.81, 1.20	0.85
rs12255372	201	228	86	0.88	0.65, 1.17	0.37	0.85	0.68, 1.07	0.17
UCP2 rs660339	203	232	79	1.19	0.91, 1.55	0.20	0.93	0.77, 1.12	0.42

Abbreviations: CI, confidence interval; LRT, likelihood ratio test; RR, relative risk.
[a] Results are based on an additive model (i.e., the risk of being a heterozygote vs. the common homozygote).

clear that *TCF7L2* is not only a key determinant of β-cell mass in the pancreas but is also essential for maintaining the secretory function in mature β cells and glucose homeostasis (45, 54–57). SNP rs3814573 was found to be associated with both type 2 diabetes risk and age of onset in Mexican Americans (58). In our study, we observed a maternal effect of the rs3814573 T allele and increased NTD risk. When we stratified by maternal obesity status, the relative risk was higher among obese women than among nonobese women. This finding suggests that the maternal genetic effect of the *TCF7L2* rs3814573 genotype on NTD risk may be different between obese women and nonobese women. The estimated relative risk remained the same when the analysis was restricted to nondiabetic women (data not shown).

Leptin is a hormone that is produced and secreted by white adipose tissue and has profound effects on eating behavior, metabolic rate, endocrine axes, and glucose homeostasis. Leptin deficiency in both mice and humans causes morbid obesity and diabetes, and replacement treatment leads to decreased food intake, normalized glucose homeostasis, and increased energy expenditure (59). In a previous study, Shaw et al. (60) reported a modest increase in spina bifida risk among infants carrying 2 genetic markers adjacent to the *LEP* gene irrespective of maternal body mass index. In this study, we observed a modest increase in NTD risk among women who carried the risk allele of SNP rs2071045. There appeared to be differences in effect between obese and nonobese women for selected *LEP* SNPs. For instance, we observed a positive association between offspring genotypes of *LEP* rs3828942 and rs12706831 and NTD risk among nonobese women, whereas in obese women, the minor allele was protective. Our findings add evidence in support of the hypothesis that

leptin/leptin receptor signaling is involved in maternal obesity-related NTD risk (25, 26, 60).

Uncoupling proteins are mitochondrial membrane transporters that play an important role in the pathogenesis of various metabolic disorders, including obesity and diabetes (61, 62). The nonsynonymous variant of the uncoupling protein 2 gene (*UCP2*), Ala55Val (rs660339), has been associated with body fat distribution and obesity (63, 64). Our colleagues previously reported a 2-fold increase in NTD risk among infants who carried the risk allele at rs660339 in a California population (30). However, this association was not observed in an Irish population (65). Our current data revealed a modest increase in NTD risk among infants who carried the risk allele, which is consistent with our previous finding. Additionally, the offspring genetic effect was greater among obese women than among nonobese women.

An important strength of our study was the use of data from the NBDPS, the largest population-based study of birth defects to be conducted to date, which provided us with a unique opportunity to examine both maternal and offspring genetic effects on NTD risk. We employed a case-parent triad design, which is immune to confounding by race/ethnicity (i.e., population stratification) in the assessment of offspring genotypes (37). Additionally, we restricted our analyses to non-Hispanic whites to limit population stratification in the assessment of maternal genetic effects. The log-linear modeling approach to analyses also allowed us to include data from incomplete triads (i.e., genotype data were missing for one or two individuals) (40, 66). An additional strength of the NBDPS is the extensive and standardized case review employed by clinical geneticists, which maximizes homogeneity among case groups. The main weakness of this study was the limited proportion of families with biologic samples available because of the generally low participation rates for contributing biologic samples (49%). Additionally, we did not conduct haplotype association analyses, because the SNPs selected for this study were primarily those identified as being associated with diabetes and/or obesity risk or because they were functional variants in these candidate genes, rather than haplotype tagging SNPs. The low percentage of families on which the genetic findings were based could limit our ability to generalize these results. However, we do not think that the demographic differences between persons who were included in this study and those who were not included were associated with genotypes.

In conclusion, our findings suggest that genetic variants associated with glucose metabolism may modify a woman's risk of having an NTD-affected pregnancy. The maternal effects of *FTO*, *TCF7L2*, and *LEP* genes may also provide evidence regarding the molecular mechanisms underlying the development NTDs. Replication of these findings in other populations and investigation of additional genes is warranted. Furthermore, since maternal obesity and diabetes are also risk factors for other malformations (5, 8, 67), assessing the association between these variants and other birth defects will broaden our understanding of diabetes and obesity-related teratogenicity.

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Author affiliations: Human Genetics Center, Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas, Houston, Texas (Philip J. Lupo, A. J. Agopian, Laura E. Mitchell); Birth Defects Epidemiology and Surveillance Branch, Texas Department of State Health Services, Austin, Texas (Mark A. Canfield); Dell Pediatric Research Institute, Department of Nutritional Sciences, University of Texas at Austin, Austin, Texas (Claudia Chapa, Wei Lu, Richard H. Finnell, Huiping Zhu); Department of Pediatrics, School of Medicine, Stanford University, Stanford, California (Gary M. Shaw); Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas, Houston, Texas (D. Kim Waller); and Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina (Andrew F. Olshan).

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