Examination of Type 2 Diabetes Loci Implicates *CDKAL1* as a Birth Weight Gene

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OBJECTIVE—A number of studies have found that reduced birth weight is associated with type 2 diabetes later in life; however, the underlying mechanism for this correlation remains unresolved. Recently, association has been demonstrated between low birth weight and single nucleotide polymorphisms (SNPs) at the *CDKAL1* and *HHEX-IDE* loci, regions that were previously implicated in the pathogenesis of type 2 diabetes. In order to investigate whether type 2 diabetes risk–conferring alleles associate with low birth weight in our Caucasian childhood cohort, we examined the effects of 20 such loci on this trait.

RESEARCH DESIGN AND METHODS—Using data from an ongoing genome-wide association study in our cohort of 5,465 Caucasian children with recorded birth weights, we investigated the association of the previously reported type 2 diabetes–associated variation at 20 loci including *TCF7L2*, *HHEX-IDE*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, *CDKAL1*, *CDKN2A/2B*, and *JAZF1* with birth weight.

RESULTS—Our data show that the minor allele of rs7756992 ($P = 8 \times 10^{-5}$) at the *CDKAL1* locus is strongly associated with lower birth weight, whereas a perfect surrogate for variation previously implicated for the trait at the same locus only yielded nominally significant association ($P = 0.01$; r^2 rs7756992 = 0.677). However, association was not detected with any of the other type 2 diabetes loci studied.

CONCLUSIONS—We observe association between lower birth weight and type 2 diabetes risk–conferring alleles at the *CDKAL1* locus. Our data show that the same genetic locus that has been identified as a marker for type 2 diabetes in previous studies also influences birth weight. ***Diabetes*** **58:2414–2418, 2009**

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It has been reported that reduced birth weight is associated with an increased risk of type 2 diabetes later in life (1–3). The largest such study was a meta-analysis of 14 studies involving a total of 132,180 individuals that demonstrated an association between lower birth weight and type 2 diabetes risk with an odds ratio of 1.32 (2). On a global level, reduced birth weight has been shown to be correlated with increased type 2 diabetes risk in 28 of 31 populations studied (3). Furthermore, low birth weight has been associated with both type 2 diabetes ($P = 0.008$) and impaired insulin secretion ($P = 0.04$) in 2,003 participants from the Helsinki Birth Cohort Study (HBCS) (4).

It has been proposed that the relationship between low birth weight and type 2 diabetes is genetically mediated, namely, the fetal insulin hypothesis (5,6). Because insulin is a key fetal growth factor, the genetic variants that reduce insulin secretion or insulin sensitivity might also reduce birth weight as well as increase the risk of developing type 2 diabetes later in life (5,6).

Studies of monogenic diabetes support the fetal insulin hypothesis where gene mutations such as *GCK*, *INS*, *INSR*, and *KCNJ11* have been shown to track with both low birth weight and diabetes (5,7,8). It has also been shown from epidemiological studies that paternal genetic contributions can directly predispose the offspring to general type 2 diabetes through reduced birth weight (9), whereas the maternal genetic contribution to the trait is less clear because it is more difficult to separate the influence of genes transferred from mother to offspring from that of the maternal environment (which in turn may be influenced by the mother's own genes) (10,11).

Recent genome-wide association (GWA) studies of type 2 diabetes have revealed a number of loci (12–22), some of which have been subsequently explored in the context of birth weight. In the HBCS study, the type 2 diabetes risk–conferring allele in *HHEX-IDE* yielded a trend toward low birth weight, whereas the equivalent allele at the *CDKN2A/2B* locus was associated with high birth weight; in addition, risk variants at *HHEX-IDE*, *CDKN2A/2B*, and *JAZF1* genes were shown to interact with birth weight but not *TCF7L2*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, and *CDKAL1*. Indeed, the highest risk of going on to develop type 2 diabetes was among the lower birth weight participants carrying the implicated risk variants (4). More recently, examination in four studies of Caucasian Europeans consisting of 7,986 mothers and 19,200 offspring of the five type 2 diabetes genes *CDKAL1*, *CDKN2A/2B*, *HHEX-IDE*, *IGF2BP2*, and *SLC30A8* with

TABLE 1
Quantitative association results for previously studied type 2 diabetes risk alleles with birth weight in the European American cohort (n = 5,465), sorted by chromosomal location

Chromosome	SNP	Minor allele	MAF	BP	Nearby gene	n	β	SE	r^2	T	P
3	rs17793693	A*	0.09634	12320971	*PPARG*	5,465	0.04854	0.03254	0.0004072	1.492	0.1358
3	rs6802898	T*	0.1212	12366207	*PPARG*	5,460	0.03545	0.02948	0.0002648	1.202	0.2293
3	rs4402960	T	0.3263	186994389	*IGF2BP2*	5,461	0.01568	0.02017	0.0001107	0.7774	0.4369
6	rs4712523	G	0.3204	20765543	*CDKAL1*	5,465	−0.05303	0.02068	0.001202	−2.564	0.01037
6	rs7756992**	G	0.2794	20787688	*CDKAL1*	5,464	−0.08449	0.0214	0.002846	−3.948	7.97×10^{-5}
7	rs1635852	T*	0.4941	27962651	*JAZF1*	5,464	0.007681	0.01921	2.93E-05	0.3998	0.6893
8	rs13266634	T*	0.2969	118253964	*SLC30A8*	5,460	0.01721	0.02102	0.0001228	0.8189	0.4129
9	rs2383207	G*	0.4583	22105959	*CDKN2A/B*	5,465	0.003944	0.01933	7.63E-06	0.2041	0.8383
10	rs1111875	T*	0.4027	94452862	*HHEX-IDE*	5,465	−0.004147	0.01949	8.29E-06	−0.2128	0.8315
10	rs7923837	A*	0.3822	94471897	*HHEX-IDE*	5,465	−0.005545	0.01967	1.46E-05	−0.2819	0.778
10	rs7903146	T	0.3057	114748339	*TCF7L2*	5,465	−0.007205	0.02069	2.22E-05	−0.3482	0.7277
11	rs1557765	T	0.3685	17360215	*KCNJ11*	5,457	0.002475	0.0199	2.84E-06	0.1244	0.901

The direction of effect is shown for the minor allele in each case. *Major allele previously reported to be associated with type 2 diabetes; **$P \leq 0.002$. β, regression coefficient for the test SNP; BP, base pair position; MAF, minor allele frequency; n, number of subjects tested; P, two-sided trend test P value; r^2, value in linear regression; T, test statistic.

lower birth weight revealed strong association with *CDKAL1* and *HHEX-IDE* when inherited by the fetus but not for *CDKN2A/2B*, *IGF2BP2*, and *SLC30A8* (6).

In this study, we sought to clarify these reported associations between low birth weight and type 2 diabetes loci using data from an ongoing GWA study in a cohort of 5,465 European American children with recorded birth weights. The criteria for locus selection were that they either came directly from published type 2 diabetes GWA studies or were type 2 diabetes genes found through the candidate gene approach that have also been reported to be associated with birth weight previously. We queried for known variants at the type 2 diabetes–associated loci of *TCF7L2*, *HHEX-IDE*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, *CDKAL1*, *CDKN2A/2B*, and *JAZF1* with respect to their correlation with birth weight to directly compare and contrast with what was recently reported by two European groups (4,6). We also queried for an additional 11 established type 2 diabetes loci that have not been previously reported with respect to birth weight including *MNTR1B*, which was first implicated in multiple GWA studies of the related trait of fasting glucose and was subsequently associated with type 2 diabetes within the same studies (15,17,22).

RESEARCH DESIGN AND METHODS

Research subjects

European American Pediatric cohort from Philadelphia. All subjects were consecutively recruited from the greater Philadelphia area from 2006–2009 at the Children's Hospital of Philadelphia. Our study cohort consisted of 5,465 singleton children of European ancestry with recorded birth weight information. We did not observe a cohort effect or temporal trends in the data. All of these participants had their blood drawn in an 8-ml ethylenediamine tetraacetic acid blood collection tube and subsequently DNA extracted for genotyping. All subjects were biologically unrelated and were aged 0–21 years. This study was approved by the Institutional Review Board of The Children's Hospital of Philadelphia. Parental informed consent was given for each study participant for both the blood collection and subsequent genotyping.

Genotyping

Illumina Infinium assay. We performed high-throughput genome-wide single nucleotide polymorphism (SNP) genotyping using the Illumina Infinium II HumanHap550 or Human 610 BeadChip technology (Illumina, San Diego, CA) at The Children's Hospital of Philadelphia's Center for Applied Genomics, as described previously (23). The SNPs analyzed survived the filtering of the genome-wide dataset for SNPs with call rates <95%, minor allele frequency <1%, missing rate per person >2%, and Hardy-Weinberg equilibrium $P < 10^{-5}$.

Most loci described from GWA studies published to date have been found using either the Affymetrix or Illumina platform. In the event a locus was reported using both the Illumina and Affymetrix arrays, we used the SNPs present on the Illumina array. In the event of a signal only being described on the Affymetrix array, we either already had that SNP on our Illumina array or identified and used the best surrogate SNP available based on the CEU HapMap (supplementary Table 1, available in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0506/DC1). We used two SNPs at the *CDKAL1* (rs4712523 and rs7756992; r^2 = 0.677), *HHEX-IDE* (rs1111875 and rs7923837; r^2 = 0.698), and *PPARG* (rs17793693 and rs6802898; r^2 = 0.011) loci as the association with type 2 diabetes, taken from various GWA studies that reported various SNPs that were in imperfect linkage disequilibrium with each other. In addition, rs4712523 is a proxy (r^2 = 1) for rs10946398, which was previously associated with birth weight.

Analysis

Normalization of birth weight data. From our database, we eliminated outliers with birth weight <1 or >8 kg, i.e., those individuals not within the credible range for birth weight at term, to avoid the potential consequences of error or Mendelian causes of extreme birth weight. Each birth weight value was adjusted for each sex separately then expressed as a z score.

Association. We queried the data for the SNPs of interest in our pediatric sample. All statistical analyses were carried out using the software package PLINK v. 1.05 (24). Ethnicity for our cohort was derived using the multidimensional scaling feature within PLINK. By treating birth weight as a quantitative trait (treated as a z score after correcting for sex), association analysis for each SNP was carried out using linear regression analysis with the SNP included as an independent variable (coded as 0, 1, and 2). With 5,465 subjects, the powers to detect 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1% variation at the P = 0.002 level (i.e., the corrected P value for the number of tests) were 47.4, 74.6, 90.0, 96.6, 98.9, 100, and 100%, respectively.

RESULTS

In our initial analysis, 12 SNPs corresponding to the 9 type 2 diabetes loci previously studied in the context of birth weight were investigated in our cohort, namely, *TCF7L2*, *HHEX-IDE*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, *CDKAL1*, *CDKN2A/2B*, and *JAZF1* (4,6) (Table 1).

As a result, we observed strong association with rs7756992 ($P = 8 \times 10^{-5}$) at the *CDKAL1* locus with low birth weight; this SNP yielded strongest association to type 2 diabetes in an Icelandic GWA study carried out on the Illumina HumanHap500 platform (21). SNPs rs10946398 or rs7754840 at the same locus have been reported to be most strongly associated with type 2 diabetes from GWA studies on the Affymetrix platform or the Illumina HumanHap300 BeadChip (16,18,19); however, using a perfect surrogate, rs4712523 (r^2 = 1), we only observed nominally significant association (P = 0.01). It

should be noted that rs10946398 and rs7756992 are far from being in perfect linkage disequilibrium ($r^2 = 0.677$), thus the inclusion of both in this current study.

Unlike previous reports, we did not observe association between rs1111875 at the *HHEX-IDE* locus and this trait (6). In line with previous reports, we also did not observe association between birth weight and *TCF7L2*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, *CDKN2A/2B*, or *JAZF1* (4,6,10).

Furthermore, we did not observe any significant association with risk alleles at other type 2 diabetes loci after correction for multiple testing for all 23 SNPs (threshold $P \leq 0.002$) (supplementary Table 2). We detected nominal association with rs1387153 ($P = 0.02$) at the *MTNR1B* locus; however, the corresponding type 2 diabetes risk allele was tracking with higher birth weight. We also analyzed male and female subjects separately, but the effect of each locus on birth weight did not vary by sex (supplementary Tables 3 and 4).

DISCUSSION

From this interim analysis of our ongoing GWA study of birth weight in a European American cohort, it is clear that the *CDKAL1* locus, which was uncovered in GWA analyses of type 2 diabetes, is strongly associated with birth weight in our study population. This result clearly supports a previous report that came to a similar conclusion (6). However, the study by Freathy et al. used a different SNP, namely, rs10946398, which was not present on our Illumina BeadChip; we used a perfect surrogate, i.e., rs4712523 ($r^2 = 1$), that only yielded nominal significance ($P = 0.01$). Although they did not report for rs7756992, we found that it gave us the strongest association ($P = 8 \times 10^{-5}$) and was selected for this study because it yielded the strongest association to type 2 diabetes in an Icelandic GWA study (21).

Secondly, we did not observe association between *HHEX-IDE* and birth weight, which is in contrast with what had been described previously (6). We acknowledge that our cohort is smaller than the original report (5,465 vs. 19,200 individuals); indeed, this association was not observed ($P < 0.05$) in the similarly sized 1958 birth cohort (6). The lack of available covariate data, such as gestational age, was also a limitation of this study. Therefore, it is possible that with a larger cohort with additional covariate data we may observe the association of this locus with birth weight; however, it could also indicate that *HHEX-IDE* has a less pronounced impact on birth weight than *CDKAL1*.

Consistent with the existing literature, we did not find any evidence of association between birth weight and *TCF7L2*, *PPARG*, *KCNJ11*, *SLC30A8*, *IGF2BP2*, *CDKN2A/2B*, or *JAZF1* (4,6,10). Given the monogenic precedent for opposing effects of maternal and fetal genotype (25), it is possible that effects of common type 2 diabetes alleles could be masked by this phenomenon.

The exact function of *CDKAL1* is unknown. It has been shown that *CDKAL1* is expressed in the rat pancreatic β-cell line Ins-1 (21). Homozygous carriers of the risk allele have been shown to have a 22% lower corrected insulin response than individuals who are wild-type carriers. It has been suggested that *CDKAL1* might influence the secretion of insulin by interacting with *CDK5* (21). Our data contributes another piece of evidence supporting the hypothesis, namely, that the same genotype conferring lower birth weight can also confer higher type 2 diabetes risk later in life. *CDKAL1* was first described in the context of type 2 diabetes in both European Caucasians and in Han Chinese (21); as such, it would be interesting to examine whether the association of *CDKAL1* with lower birth weight also stands in this and other ethnicities, such as African Americans and Hispanics.

In conclusion, we strongly confirm that the established type 2 diabetes locus *CDKAL1* also influences birth weight. However, we do not observe such association with *TCF7L2*, *HHEX-IDE*, *CDKN2A/2B*, or *JAZF1*. In addition, of all the other established type 2 diabetes loci to date, we do not observe a convincing role for them in the determination of birth weight.

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No potential conflicts of interest relevant to this article were reported.

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