Examination of type 2 diabetes loci implicates CDKAL1 as a birth weight gene

Jianhua Zhao¹, Mingyao Li², Jonathan P. Bradfield³, Kai Wang³, Haitao Zhang³, Patrick Sleiman³, Cecilia E. Kim³, Kiran Annaiah³, Wendy Glaberson³, Joseph T. Glessner³, George F. Otieno³, Kelly A. Thomas³, Maria Garris³, Cuiping Hou³, Edward C. Frackleton³, Rosetta M. Chiavacci³, Robert I. Berkowitz⁴,⁵, Hakon Hakonarson¹,³,⁶* and Struan F.A. Grant¹,³,⁶*

¹Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA; ²Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; ³Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA; ⁴Behavioral Health Center and Department of Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, Philadelphia PA 19104, USA; ⁵Center for Weight and Eating Disorders, Department of Psychiatry, University of Pennsylvania, Philadelphia PA 19104, USA; ⁶Department of Pediatrics, University of Pennsylvania, Philadelphia PA 19104, USA

*To whom correspondence should be addressed.
Struan F.A. Grant
E-mail: grants@chop.edu
or
Hakon Hakonarson
Email: hakonarson@chop.edu

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

Submitted 6 April 2009 and accepted 29 June 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
**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 was demonstrated between low birth weight and single nucleotide polymorphisms (SNPs) at the *CDKAL1* and *HHEX-IDE* loci, regions which have been 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.

**Design and Methods:** Utilizing data from an ongoing GWA 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, JAZF1* with birth weight.

**Results:** Our data show that the minor allele of rs7756992 (*P*=8×10^{-5}) at the *CDKAL1* locus is strongly associated with lower birth weight while a perfect surrogate for variation previously implicated for the trait at the same locus only yielded nominally significant association (*P*=0.01; *r*^2^ to 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 shows that the same genetic locus that has been identified as a marker for type 2 diabetes in previous studies also influences birth weight.
It has been reported that reduced birth weight is associated with an increased risk of type 2 diabetes (T2D) later in life (1-3). The largest such study was a meta-analysis of fourteen studies involving a total of 132,180 individuals which demonstrated an association between lower birth weight and T2D risk with an odds ratio of 1.32 (2). On a global level, reduced birth weight has been shown to be correlated with increased T2D risk in 28 of 31 populations studied (3). Furthermore, low birth weight has been associated with both T2D (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 T2D is genetically mediated, namely “the fetal insulin hypothesis” (5; 6). Since 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 of T2D 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 T2D through reduced birth weight (9) while the maternal genetic contribution to the trait is less clear as 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 be the mother’s own genes) (10; 11).

Recent genome wide association (GWA) studies of T2D 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 T2D risk-conferring allele in HHEX yielded a trend towards low birth weight while 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 T2D was among the lower birth weight participants carrying the implicated risk variants (4). More recently, examination in four studies of white Europeans consisting of 7,986 mothers and 19,200 offspring of the five T2D genes CDKAL1, CDKN2A/2B, HHEX-IDE, IGF2BP2 and SLC30A8 with 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 T2D loci utilizing data from an ongoing GWA study in a cohort of 5,737 European American children with recorded birth weights. The criteria for locus selection was that they either came directly from published T2D GWA studies or were T2D 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 T2D-associated loci of TCF7L2, HHEX-IDE, PPARG, KCNJ11, SLC30A8, IGF2BP2, CDKAL1, CDKN2A/2B and JAZF1 with respect to their correlation with birth weight in order to directly compare and contrast with what was recently reported by two European groups (4; 6). We also queried for an additional 11 established T2D 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 T2D within the same studies (15; 17; 22).

MATERIAL AND METHODS

Research Subjects. Childhood European American Cohort from Philadelphia: All subjects were consecutively recruited from the Greater Philadelphia area from 2006 to 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 to an 8ml EDTA blood collection tube and were subsequently DNA extracted for genotyping. All subjects were biologically unrelated and were aged between 0 and 21 years old. 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 SNP genotyping, using the Illumina Infinium™ II HumanHap550 or Human 610 BeadChip technology (Illumina, San Diego), 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 we 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). We utilized 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 T2D from various GWA studies reported different SNPs that were in imperfect LD 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 < 1kg or > 8kg, 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 version 1.05(24). Ethnicity for our cohort was derived using the MDS feature within PLINK. By treating birth weight as a quantitative trait (treated as a z-score after correcting for gender), association analysis for each SNP was carried out using linear regression with the SNP included as an independent variable (coded as 0, 1, and 2). With 5465 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, twelve SNPs corresponding to the nine T2D 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 ×10⁻⁵) at the CDKAL1 locus with low birth weight; this SNP yielded strongest association to T2D in an Icelandic GWA study carried out on the Illumina HumanHap 500 platform (21). SNPs rs10946398 or rs7754840 at the same locus have been reported to be most strongly associated with T2D from GWA studies on the Affymetrix platform or the Illumina HumanHap 300 BeadChip (16;18;19); however using a perfect surrogate, rs4712523 (r²=1) that only yielded nominal significance (P=0.01). While they did not report for rs7756992, we found that it gave us the strongest association (P=8 ×10⁻⁵) and was selected for this study because it yielded the strongest association to T2D 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 versus 19,200 individual); 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 and JAZF1 (4;6;10).

**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 T2D, 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 Freathy et al study utilized a different SNP, namely rs10946398, which was not present on our Illumina BeadChip; we used a perfect surrogate i.e. rs4712523 (r²=1) that only yielded nominal significance (P=0.01). While they did not report for rs7756992, we found that it gave us the strongest association (P=8 ×10⁻⁵) and was selected for this study because it yielded the strongest association to T2D in an Icelandic GWA study (21).

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 and 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≤0.002). (Supplementary Table 2). We detected nominal association with rs1387153 (P=0.02) at the MTNR1B locus; however the corresponding T2D risk allele was tracking with higher birth weight. We also analyzed males and females separately but the effect of each locus on birth weight did not vary by gender (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
individuals who are wild type carriers. It has been suggested that \textit{CDKAL1} might influence the secretion of insulin by interacting with \textit{CDK5}\cite{21}. Our data contributes another piece of evidence supporting the hypothesis, namely that the same genotype conferring lower birth weight can also confer higher T2D risk later in life. \textit{CDKAL1} was first described in the context of T2D in both European Caucasians and in Han Chinese\cite{21}; as such, it would be interesting to examine whether the association of \textit{CDKAL1} with lower birth weight also stands in this and other ethnicities.

In conclusion, we strongly confirm that the established T2D locus, \textit{CDKAL1}, also influences birth weight. However we do not observe such association with \textit{TCF7L2}, \textit{HHEX-IDE}, \textit{CDKN2A/2B} or \textit{JAZF1}. In addition, of all the other established T2D loci to date, we do not observe a convincing role for them in the determination of birth weight.

**ACKNOWLEDGEMENTS**

We would like to thank all participating subjects and families. Elvira Dabaghyan, Hope Thomas, Kisha Harden, Andrew Hill, Kenya Fain, Crystal Johnson-Honesty, Cynthia Drummond, Shanell Harrison and Sarah Wildrick provided expert assistance with genotyping or data collection and management. We would also like to thank Smari Kristinsson, Larus Arni Hermannsson and Asbjörn Krisbjörnsson of Raförnin ehf for their extensive software design and contribution. This research was financially supported by the Children’s Hospital of Philadelphia.

The study is supported in part by a Research Development Award from the Cotswold Foundation (H.H. & S.F.A.G.) and NIH grant 1R01HD056465-01A1.

**Author information.** The authors declare no competing financial interests.
REFERENCES


21. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, 
Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, 
V, Chen G, Huang H, Lashley K, Doumatay A, So WY, Ma RC, Andersen G, Borch-Johnsen K, 
Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, 
Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong 
*Nat Genet* 39:770-775, 2007

Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler 
E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, 
Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, 
Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, 
Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, 
Naitza S, Orru M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Schaefer P, Scott LJ, 
Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, 
Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemse G, 
Wittekindt JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Ueda M, 
CS, Peltonen L, Groop LC, Moosey VP, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, 
Van Duij J, Duw J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, 
Abecasis GR: Variants in MTNR1B influence fasting glucose levels. *Nat Genet* 41:77-81, 2009

Casalunovo T, Taback SP, Frackelton EC, Lawson ML, Robinson LJ, Skraban R, Lu Y, 
Chivacci RM, Stanley CA, Kirsch SE, Rappaport EF, Orange JS, Monos DS, Devoto M, Qu H-Q, 
Pochronakos C: A genome-wide association study identifies KIAA0350 as a type 1 diabetes 

24. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de 
Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-

25. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: Mutations in the 
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

NMISS: number of individuals tested; BETA: regression coefficient for the test SNP; SE: standard error of the regression coefficient; R2: $r^2$ value in linear regression; T: test statistic; P: two-sided trend test $P$-value. The direction of effect is shown for the minor allele in each case.

*Major allele previously reported to be associated with T2D; ** $P \leq 0.002$

<table>
<thead>
<tr>
<th>CHR</th>
<th>SNP</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>BP</th>
<th>Nearby Gene</th>
<th>NMISS</th>
<th>BETA</th>
<th>SE</th>
<th>R2</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rs17793693</td>
<td>A*</td>
<td>0.09634</td>
<td>12320971</td>
<td>PPARG</td>
<td>5465</td>
<td>0.04854</td>
<td>0.03254</td>
<td>0.0004072</td>
<td>1.492</td>
<td>0.1358</td>
</tr>
<tr>
<td>3</td>
<td>rs6802898</td>
<td>T*</td>
<td>0.1212</td>
<td>12366207</td>
<td>PPARG</td>
<td>5460</td>
<td>0.03545</td>
<td>0.02948</td>
<td>0.0002648</td>
<td>1.202</td>
<td>0.2293</td>
</tr>
<tr>
<td>3</td>
<td>rs4402960</td>
<td>T</td>
<td>0.3263</td>
<td>186994389</td>
<td>IGF2BP2</td>
<td>5461</td>
<td>0.01568</td>
<td>0.02017</td>
<td>0.0001107</td>
<td>0.7774</td>
<td>0.4369</td>
</tr>
<tr>
<td>6</td>
<td>rs4712523</td>
<td>G</td>
<td>0.3204</td>
<td>20765543</td>
<td>CDKAL1</td>
<td>5465</td>
<td>-0.05303</td>
<td>0.02068</td>
<td>0.001202</td>
<td>-2.564</td>
<td>0.01037</td>
</tr>
<tr>
<td>6</td>
<td>**rs7756992</td>
<td>G</td>
<td>0.2794</td>
<td>20787688</td>
<td>CDKAL1</td>
<td>5464</td>
<td>-0.08449</td>
<td>0.0214</td>
<td>0.002846</td>
<td>-3.948</td>
<td>7.97E-05</td>
</tr>
<tr>
<td>7</td>
<td>rs1635852</td>
<td>T*</td>
<td>0.4941</td>
<td>27962651</td>
<td>JAZF1</td>
<td>5464</td>
<td>0.007681</td>
<td>0.01921</td>
<td>2.93E-05</td>
<td>0.3998</td>
<td>0.6893</td>
</tr>
<tr>
<td>8</td>
<td>rs13266634</td>
<td>T*</td>
<td>0.2969</td>
<td>118253964</td>
<td>SLC30A8</td>
<td>5460</td>
<td>0.01721</td>
<td>0.02102</td>
<td>0.0001228</td>
<td>0.8189</td>
<td>0.4129</td>
</tr>
<tr>
<td>9</td>
<td>rs2383207</td>
<td>G*</td>
<td>0.4583</td>
<td>22105959</td>
<td>CDKN2A/B</td>
<td>5465</td>
<td>0.003944</td>
<td>0.01933</td>
<td>7.63E-06</td>
<td>0.2041</td>
<td>0.8383</td>
</tr>
<tr>
<td>10</td>
<td>rs1111875</td>
<td>T*</td>
<td>0.4027</td>
<td>94452862</td>
<td>HHEX-IDE</td>
<td>5465</td>
<td>-0.004147</td>
<td>0.01949</td>
<td>8.29E-06</td>
<td>-0.2128</td>
<td>0.8315</td>
</tr>
<tr>
<td>10</td>
<td>rs7923837</td>
<td>A*</td>
<td>0.3822</td>
<td>94471897</td>
<td>HHEX-IDE</td>
<td>5465</td>
<td>-0.005545</td>
<td>0.01967</td>
<td>1.46E-05</td>
<td>-0.2819</td>
<td>0.778</td>
</tr>
<tr>
<td>10</td>
<td>rs7903146</td>
<td>T</td>
<td>0.3057</td>
<td>114748339</td>
<td>TCF7L2</td>
<td>5465</td>
<td>-0.007205</td>
<td>0.02069</td>
<td>2.22E-05</td>
<td>-0.3482</td>
<td>0.7277</td>
</tr>
<tr>
<td>11</td>
<td>rs1557765</td>
<td>T</td>
<td>0.3685</td>
<td>17360215</td>
<td>KCNJ11</td>
<td>5457</td>
<td>0.002475</td>
<td>0.0199</td>
<td>2.84E-06</td>
<td>0.1244</td>
<td>0.901</td>
</tr>
</tbody>
</table>