BMI at Age 8 Years Is Influenced by the Type 2 Diabetes Susceptibility Genes HHEX-IDE and CDKAL1

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OBJECTIVE—To determine whether HHEX-IDE and CDKAL1 genes, which are associated with birth weight and susceptibility to type 2 diabetes, continue to influence growth during childhood.

RESEARCH DESIGN AND METHODS—BMI, weight, and height at age 8 years expressed as age- and sex-corrected standard deviation scores (SDS) against national reference data and single-nucleotide polymorphism genotyping of HHEX-IDE and CDKAL1 loci were analyzed in 646 prospectively followed children in the German BABYDIAB cohort. All children were singleton full-term births; 386 had mothers with type 1 diabetes, and 260 had fathers with type 1 diabetes and a nondiabetic mother.

RESULTS—Type 2 diabetes risk alleles at the HHEX-IDE locus were associated with reduced BMI-SDS at age 8 years (0.17 SDS per allele; \( P = 0.004 \)). After stratification for birth weight, both HHEX-IDE and CDKAL1 risk alleles were associated with reduced BMI-SDS (0.45 SDS, \( P = 0.0002 \); 0.52 SDS, \( P = 0.0001 \)) and weight-SDS (0.22 SDS, \( P = 0.04 \); 0.56 SDS, \( P = 0.0002 \)) in children born large for gestational age (>90th percentile) but not children born small or appropriate for gestational age. Within children born large for gestational age, BMI and weight decreased with each additional type 2 diabetes risk allele (~2 kg per allele; >8 kg overall). Findings were consistent in children of mothers with type 1 diabetes (\( P < 0.0001 \)) and children of nondiabetic mothers (\( P = 0.008 \)).

CONCLUSIONS—The type 2 diabetes susceptibility alleles at HHEX-IDE and CDKAL1 loci are associated with low BMI at age 8 years in children who were born large for gestational age. Diabetes 59:2063–2067, 2010
The association BMI-SDS and birth weight was examined using linear regression, with birth weight coded as SGA (small for gestational age; <10th percentile), AGA (appropriate for gestational age; 10th–90th percentile), or LGA (large for gestational age; >90th percentile). 

§The association BMI-SDS and genotype for each SNP was examined using linear regression, with genotypes coded as 0, 1, or 2 risk alleles. 

†Adjusted for proband (children who have a mother with type 1 diabetes or children without a mother with type 1 diabetes).
homozygous for the susceptibility alleles. Per risk allele, the BMI-SDS was lower by 0.45 SDS (95% CI, 0.22–0.69) for the *HHEX-IDE* gene and by 0.52 SDS (95% CI, 0.26–0.78) for the *CDKAL1* gene in the LGA children. For both the *HHEX-IDE* and the *CDKAL1* genes, a mean BMI above the German reference population mean at 8 years was observed only in the subgroup of children who were LGA and not homozygous for the type 2 diabetes susceptibility alleles. A similar relationship was observed between the *HHEX-IDE* and the *CDKAL1* genes and weight but not height (supplementary Tables S4 and S5, available in an online appendix). No relationship between alleles of the *SLC30A8* gene and BMI-SDS were observed with or without stratification for birth weight (data not shown).

Within the LGA group, both genes were associated with BMI at age 8 years (*HHEX-IDE*, $P = 0.0001$; *CDKAL1*, $P = 0.0002$) with an adjusted $R^2$ of 0.21 in the joint regression model. Thus, the combination of alleles from both genes showed a dramatic association between the number of type 2 diabetes susceptibility alleles and lower BMI in LGA children (Fig. 2, supplementary Figure S2A and supplementary Table S6, available in an online appendix). LGA children with no type 2 diabetes susceptibility alleles at the *HHEX-IDE* and *CDKAL1* loci had BMIs almost two SDS above the population average, whereas children who had four susceptibility alleles had BMIs that were at or below the population average. Across all birth weight strata, an increased mean 8-year BMI was only seen in children who were LGA and had two or less of the type 2 diabetes susceptibility alleles for *HHEX-IDE* and *CDKAL1*. These findings were consistent in children who had a mother with type 1 diabetes ($P < 0.0001$) and children who had a father with type 1 diabetes and a nondiabetic mother ($P = 0.008$), and no evidence of interaction between maternal diabetes status and genotype on 8-year BMI was observed. Examination of weight and height in relation to these gene combinations in LGA children found an association with weight and weight-SDS ($P = 0.0002$; supplementary Figure S2B and supplementary Table S7, available in an online appendix) corresponding to a weight difference of $\sim -2$ kg per risk allele and resulting in $>8$ kg difference between LGA children with no risk alleles (mean weight, 35.7 kg) and those with all four risk alleles (mean weight, 27.5 kg). No association was observed with height-SDS ($P = 0.51$; data not shown). HOMA-IR was available at age 8 years in 63 LGA children. With this limited data, a similar, but not significant, trend was observed between the number of type 2 diabetes susceptibility alleles at the *HHEX-IDE* and *CDKAL1* loci and both fasting insulin and HOMA-IR (supplementary Figure S3, available in an online appendix).

**DISCUSSION**

An association between the recently identified type 2 diabetes susceptibility genotypes and growth during child-
hood was identified. Analysis of weight, height, and BMI in a cohort of children showed that type 2 diabetes susceptibility genotypes at the \textit{HHEX-IDE} and \textit{CDKAL1} loci were associated with reduced BMI and weight at age 8 years. The associations were interactive with birth weight status, suggesting that these genes contribute to both fetal and childhood growth patterns.

Previous studies (10–16) have shown that birth weight is associated with growth and BMI during childhood. Consistent with these studies, SGA children in our cohort were ~2 kg lighter and LGA children were 2 kg heavier than AGA children at age 8 years (data not shown). There was, however, also a very striking relationship between the \textit{HHEX-IDE} and \textit{CDKAL1} genotypes and 8-year BMI that was found in LGA children. Both genes contributed independently to reduce BMI and weight to around normal in the LGA children but not in the AGA children. Thus, our findings suggest that the type 2 diabetes susceptibility genes \textit{HHEX-IDE} and \textit{CDKAL1} influence homeostasis of body mass when there has been excessive fetal growth. Potentially relevant to this affirmation is that over half the children in our cohort were affected by maternal diabetes, a state that is associated with increased fetal growth via mechanisms that include increased fetal insulin production (17). These children are no longer exposed to diabetic glycemic variations after delivery and are therefore interesting to compare to children of nondiabetic mothers. In this regard, the associations between the \textit{HHEX-IDE} and \textit{CDKAL1} genes and 8-year BMI were consistent in LGA children from mothers with diabetes and children who had a father with type 1 diabetes and a mother without known diabetes. Therefore, although it cannot be excluded that the LGA children from the nondiabetic mothers were also exposed to hyperglycemic stimuli during fetal growth, the associations between genes and BMI appear to be independent of hyperglycemia during pregnancy, further supporting the notion that the genes influence childhood growth in addition to fetal growth.

Our findings are inconsistent with a recent report on children (18). Zhao et al. found increased BMI associated with the type 2 diabetes susceptibility allele of the \textit{HHEX-IDe} gene and no association between BMI and SNPs of the \textit{CDKAL1} gene. It should be noted that, in their study, the association between increased BMI and the \textit{HHEX-IDe} gene was only observed in children aged 2–6 years and not in older children, whereas we examined children at the age of 8 years. Moreover, inconsistent with ours and other studies (4,5), they did not find an association between the \textit{HHEX-IDe} gene and birth weight in their cohort. Finally, because our associations were limited to children who were LGA, it is possible that these would be missed in a cohort that is not overly represented by LGA children.

The relationship of our findings to type 2 diabetes susceptibility and pathogenesis is unclear. Many have shown that SGA is associated with increased type 2 diabetes risk (19–22), and some have also suggested that LGA is associated with increased risk (23–25). The relationship of type 2 diabetes susceptibility genes with growth and BMI appears complex, and there is no general rule for the direction of the effect of type 2 diabetes susceptibility and BMI. Ours and previous studies consistently show that SGA is associated with reduced weight, height, and BMI during childhood (10–16). Our data further show that having the full complement of type 2 diabetes susceptibility genotypes for two genes protects against increased BMI at age 8 years. Analogous to this, the protective variant of the type 2 diabetes susceptibility gene \textit{PPARG2} is associated with increased BMI (26–28). Thus, while type 2 diabetes is generally associated with increased BMI, there are examples where increased BMI can be associated with reduced type 2 diabetes risk. It is possible that, in such examples, large fat deposits may protect against accumulation in the liver and muscle in children with protective alleles. It is also possible that, although BMI is not increased, other characteristics such as adiposity or insulin sensitivity may be already affected in the children (29,30), or that different gene effects are seen in the subset of children with increased adiposity (28) or under certain dietary conditions such as was found for peroxisome proliferator–activated receptor-\(\gamma\) (31). Relevant to our cohort, which has an increased load of type 1 diabetes–associated genetic susceptibility, it is possible that the relative contribution of genes such as \textit{HHEX-IDe} and \textit{CDKAL1} to pathogenesis may be outweighed by a series of other genes. Finally, longer follow-up of our and, in particular, type 2 diabetes–susceptible cohorts will be required to ascertain if the association of lower weight and BMI with the type 2 diabetes susceptible alleles for the \textit{HHEX-IDe} and \textit{CDKAL1} genes persists or changes after puberty and in adulthood.

In conclusion, we show that the fetal programming associated with the type 2 diabetes susceptibility genes \textit{HHEX-IDe} and \textit{CDKAL1} has prolonged effects during childhood as evidenced by the influence of birth weight status on BMI and weight at age 8 years, and that further programming in association with these genes occurs during childhood when there has been excess fetal growth. These data indicate that there is important genetic control of childhood weight that may act through mechanisms associated with insulin release and action. The relationship of these genetic factors to weight control during childhood appears inconsistent to their association with type 2 diabetes later in life, a finding that requires validation and longer follow-up of cohorts that are susceptible for type 2 diabetes.

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\textbf{REFERENCES}

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REFERENCES

1. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PJ, Abecasis GR, Almgren P, Andersson G, Ardlie K, Boström KB, Bergman RN, Bonnycaisec L, Borich-Johansen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren WL, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hittman GA, Hughes TE, Isomaa B, Jackson AU, Jergensen T, Kong A, Kukulanza K, Kurvillina FG, Kuusisto J, Langenberg C, Lango H, Lauritzen E, Lin JP, Lindsey GM, Lin T, Liu TT, Loh PC, Lyssenko V, Marchini J, Meitinger T, Metspalu E, Metspalu M, Mieli-Vergani G, Mitchell BD, Montgomery GW, Morange P, Mosley TH, Nauck M, Nalls MA, Nilsson P, Owen RW, Palmer CN, Payne F, Perry JR, Petersen ES, Platou C, Frokovenko I, Qin L, Qin L, Rayner NW, Roes M, Roix JJ, Sandberg A, Shieh DT, Sjogren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ, Welkove TR, Witham TR, Wu CH, Xiang JK, Yusuf SS, Zellner K, Ziegler A, Hebebra J. Percentile für den Body-mass-index für deutsche Kinder und Jugendliche. http://www.mybmi.de. Accessed 18 November 2008

2. Grant RP, Nature Genet 2008;40:638–645

3. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2007;39:1035–1031

4. Fraythy RM, Bennett AJ, Ring SM, Shields B, Sjo¨ gren M, Watanabe RM, Weedon MN, Willer CJ, Welkove TR, Wu CH, Xiang JK, Yusuf SS, Zellner K, Ziegler A, Hebebra J. Percentile für den Body-mass-index für deutsche Kinder und Jugendliche. http://www.mybmi.de. Accessed 18 November 2008

5. Voigt M, Schneider KT, Jürgen K. Analysis of a 1992 birth sample in Germany. I. New percentile values of the body weight of newborn infants. Geburtshilfe Frauenheilkd 1996;56:550–558

6. Strauss RS. Effects of the intrauterine environment on childhood growth. Br Med Bull 1997;53:81–95

7. Luan J, Browne PO, Harding AH, O'Rahilly S, Chatterjee VK. Evidence for gene-nutrient interaction at the PPARγ locus. Diabetes 2001;50:686–689

8. Wareham NJ. Evidence for gene-nutrient interaction at the PPARγ locus. Diabetes 2001;50:686–689