Diabetologia (2010) 53:1908–1916
DOI 10.1007/s00125-010-1790-0

ARTICLE

Type 2 diabetes risk alleles near *ADCY5*, *CDKAL1* and *HHEX-IDE* are associated with reduced birthweight

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Received: 1 March 2010 / Accepted: 19 April 2010 / Published online: 20 May 2010

Abstract
Aims/hypothesis The fetal insulin hypothesis suggests that variation in the fetal genotype influencing insulin secretion or action may predispose to low birthweight and type 2 diabetes. We examined associations between 25 confirmed type 2 diabetes risk variants and birthweight in individuals from the Danish Inter99 population and in meta-analyses including Inter99 data and reported studies.
Methods Midwife records from the Danish State Archives provided information on mother's age and parity, as well as birthweight, length at birth and prematurity of the newborn in 4,744 individuals of the population-based Inter99 study. We genotyped 25 risk alleles showing genome-wide associations with type 2 diabetes.
Results Birthweight was inversely associated with the type 2 diabetes risk alleles of *ADCY5* rs11708067 (β=−33 g [95% CI −55, −10], p=0.004) and *CDKAL1* rs7756992 (β=−22 g [95% CI −43, −1], p=0.04). The association for the latter locus was confirmed in a meta-analysis (n=24,885) (β=−20 g [95% CI −29, −11], $p=5\times10^{-6}$). The *HHEX-IDE* rs1111875 variant showed no significant association among Danes (p=0.09); however, in a meta-analysis (n=25,164) this type 2 diabetes risk allele was associated with lower birthweight (β=−16 g [95% CI −24, −8], $p=8\times10^{-5}$). On average, individuals with high genetic risk (≥25 type 2 diabetes risk alleles) weighed marginally less at birth than those with low genetic risk (<25 type 2 diabetes risk alleles) (β=−35 g [95% CI −69, −2], p=0.037).
Conclusions/interpretation We report a novel association between the fetal *ADCY5* type 2 diabetes risk allele and decreased birthweight, and confirm in meta-analyses associations between decreased birthweight and the type 2 diabetes risk alleles of *HHEX-IDE* and *CDKAL1*. No strong general effect on birthweight can be ascribed to the 25 common type 2 diabetes risk alleles.

Keywords Birthweight · Genetic variants · Type 2 diabetes

E. A. Andersson and K. Pilgaard contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-010-1790-0) contains supplementary material, which is available to authorised users.

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Abbreviations

CEU	Centre d'Etude du Polymorphisme (Utah residents with northern and western European ancestry)
HOMA-B	HOMA of beta cell function
MAGIC	Meta-Analyses of Glucose and Insulin-Related Traits Consortium

Introduction

Previous studies have found that reduced birthweight is associated with impaired glucose tolerance and type 2 diabetes later in life [1, 2]. A recent meta-analysis of 14 studies demonstrated that the odds ratio of developing type 2 diabetes in adulthood is 1.47 for individuals born with low birthweight [3]. However, the aetiopathogenic mechanisms behind this association are far from elucidated. Twin studies have indicated a non-genetic origin of the association between birthweight and type 2 diabetes [4], and the 'thrifty phenotype hypothesis' suggests that poor nutritional conditions during critical periods of fetal life alter beta cell function and/or insulin action, thereby predisposing to type 2 diabetes in later life [2]. Insulin is important for fetal growth and for metabolism throughout life. The 'fetal insulin hypothesis' states that variation in the fetal genotype affecting pancreatic beta cell function or insulin sensitivity and thereby availability of insulin as a fetal growth factor predisposes to reduced birth size and risk of type 2 diabetes in later life [5]. This hypothesis is supported by the finding that newborns with mutations in *GCK*, *KCNJ11*, *HNF1B* and *INS* have marked insulin deficiency and considerably reduced birthweight [6–9]. In contrast, newborns with mutations in *HNF4A*, which also cause adult insulin deficiency and monogenic diabetes, have a higher birthweight due to elevated insulin secretion by the fetus [10], demonstrating that fetal and adult insulin secretion are not always correlated.

Maternal blood glucose level during pregnancy is a main determinant of fetal insulin secretion and thus fetal growth. Maternal hyperglycaemia may lead to fetal hyperinsulinaemia and consequently macrosomia is a well-known complication of untreated gestational diabetes. The maternal genotype may thereby influence fetal exposure to maternal glucose and thus interact with the effect of fetal genotype on fetal growth and subsequent birthweight of the newborn [6].

A total of 25 confirmed type 2 diabetes risk gene variants have been reported [11–31]. Of these, variants in or near *NOTCH2*, *THADA*, *PPARG*, *ADAMTS9*, *IGF2BP2*, *CDKAL1*, *JAZF1*, *SLC30A8*, *CDKN2A/B*, *CDC123*, *HHEX-IDE*, *TCF7L2*, *KCNQ1*, *KCNJ11*, *MTNR1B*, *TSPAN8*, *GCK* and *FTO* have previously been investigated in relation to birthweight [32–36]. However, only type 2 diabetes risk variants near *HHEX-IDE* and *CDKAL1* have been associated with lower birthweight [32–34]. Zhao et al. found a highly significant effect of *CDKAL1* rs7756992, but no significant association for the *HHEX-IDE* variant [34]. This is in contradiction with a Finnish cohort study, in which only an association for the *HHEX-IDE* locus was observed [33]; however, another *CDKAL1* variant (rs7754840) was investigated in the latter study. Type 2 diabetes risk variants near *TCF7L2* and *CDKN2A/B* have been associated with increased birthweight [33, 36].

Complete consistency among previous studies has not been achieved. Moreover, to our knowledge, five newly identified type 2 diabetes risk variants [13] have not been investigated in relation to fetal growth. The overall aim of this study was therefore to investigate the association between the 25 confirmed type 2 diabetes risk variants and birthweight in 4,213 individuals from the Danish Inter99 population (ClinicalTrials.gov NCT00289237) [37]. We also examined associations between type 2 diabetes risk variants and birthweight in meta-analyses including our own data and published studies.

Methods

Study population Individuals examined in the present study were from the Danish Inter99 Study, which at baseline comprised 6,784 individuals living in the region of Copenhagen. The Inter99 is a population-based randomised non-pharmacological intervention study of prevention of ischaemic heart disease conducted at the Research Centre for Prevention and Health in Glostrup, Denmark (www.inter99.dk) [37, 38]. For 4,744 participants, midwife journals were traced through the Danish State Archives. These journals contained information on mothers' age, parity and marital status as well as birthweight, length at birth and prematurity of the newborn. Ponderal index was calculated as birthweight (kg)/birth length (m^3). Information about mothers' diabetes status was obtained by a questionnaire during the baseline visits in 1999 to 2001. The age of onset of maternal diabetes was not registered.

Pregnancies were considered at term when gestation attained 36 complete weeks and did not exceed 41 complete weeks. Pre-term singleton deliveries (n=446) and individuals born from multiple pregnancies (n=85) were excluded, since these newborns are lighter presumably due to non-genetic factors. The final number of individuals included in the study was 4,213. All individuals were Danes by self-report. All participants gave

written informed consent and the protocol was in accordance with the Helsinki Declaration and approved by local ethic committees.

Genotyping Genotyping was performed using a genotyping device (KASPar; KBioscience, Hoddesdon, UK). All genotyping success rates were >96% with an error rate of <0.5%, except *ADAMTS9* rs4607103, for which the error rate was 0.9%. All genotypes obeyed Hardy–Weinberg equilibrium in the Danish population ($p>0.05$) except *HHEX-IDE* rs1111875 ($p=0.03$), *TCF7L2* rs7903146 ($p=0.02$), *DGKB/TMEM195 rs*2191349 ($p=0.01$) and *SLC30A8* rs13266634 ($p=0.009$).

Statistical analysis All statistical analyses were performed using RGui version 2.8.1 (available at http://www.r-project.org). The effect of the genetic variants on birthweight, length at birth and ponderal index in the Inter99 population was calculated using linear regression models adjusted for sex, maternal diabetes (yes vs no/not available) and parity (0, 1, 2, 3 or ≥4). No transformation of data was performed. Only additive genetic models were considered assuming a constant change in birthweight per risk allele, with $p<0.05$ being considered significant.

Statistical power was estimated using 1,000 simulations. We used the empirical variance of the observed traits adjusted for sex, maternal diabetes and parity to simulate phenotypes from a normal distribution, so that variance across genotypes is drawn from the estimated variance. In the Danish Inter99 study, we had more than 80% statistical power to detect effects of 45 g, 35 g and 30 g, assuming minor allele frequencies of 10%, 20% and 40%, respectively.

Fixed-effect meta-analyses (up to $n=25,957$) were performed using effect size estimates and standard errors derived from linear regression analyses from this and three other studies [32, 33, 36]. Weight of studies in the meta-analyses was estimated using inverse variance assuming fixed effects. Heterogeneity was measured by Q-statistics.

A combined analysis of variants within *CDKAL1*, *HHEX-IDE* and *ADCY5* loci was performed by summing up risk alleles of the three variants, where individuals can have from zero to six risk alleles.

Information from the 25 type 2 diabetes risk variants in 2,733 individuals with available genotype data on all 25 variants was combined into a binary risk allele score (low vs high genetic risk). In this population, the median number of risk alleles was 25. The combined effect was calculated by comparing two groups according to their number of risk alleles (low genetic risk, i.e. <25 type 2 diabetes risk alleles, $n=1,366$ vs high genetic risk, i.e. ≥25 type 2 diabetes risk alleles, $n=1,367$). In the combined analyses similar (fixed) effects of the variants were assumed.

Results

Quantitative analyses of birthweight in the Danish Inter99 population Characteristics of the Inter99 participants are shown in Electronic supplementary material [ESM] Table 1. The 25 confirmed type 2 diabetes risk variants were investigated for an association with birthweight in 4,213 individuals from the Danish Inter99 population and the results of the quantitative analyses are shown in Table 1. The risk allele of rs11708067 at the *ADCY5* locus was associated with a reduction in birthweight (per allele $\beta=-33$ g [95% CI −55, −10], $p=0.004$) (Table 1). Also carriers of the *CDKAL1* rs7756992 risk allele had lower birthweight (per allele $\beta=-22$ g [95% CI −43, −1], $p=0.04$) (Table 1). The risk allele of rs1111875 near *HHEX-IDE* showed a tendency towards lower birthweight (per allele $\beta=-17$ g [95% CI −36, 2], $p=0.09$) (Table 1). *ADCY5* rs11708067 was also associated with a slightly decreased ponderal index (per allele $\beta=-0.1$ kg/m^3 [95% CI −0.2, −0.02], $p=0.02$) (Table 2), while *CDKAL1* rs7756992 was associated with reduced birth length (−0.1 cm [95% CI −0.2, −0.01], $p=0.04$) (Table 2). The risk allele of *WFS* rs10010131 showed a trend towards higher birthweight (per allele $\beta=19$ g [95% CI −1, 39], $p=0.06$) (Table 1). In the Inter99 study population, none of the associations remained significant after correction for multiple testing.

Meta-analyses Meta-analyses including previously published data on type 2 diabetes loci in relation to birthweight were performed for *CDKAL1* ($n=24,885$), *HHEX-IDE* ($n=25,164$), *TCF7L2* ($n=19,745$), *SLC30A8* ($n=24,908$), *IGF2BP2* ($n=24,393$), *CDKN2A/B* ($n=25,957$), *PPARG* ($n=6,206$), *KCNJ11* ($n=6,206$) and *JAZF1* ($n=6,206$) [32, 33, 36]. No data were available for the remaining variants.

In the meta-analysis of *CDKAL1*, three different proxies in linkage disequilibrium (HapMap Centre d'Etude du Polymorphisme [Utah residents with northern and western European ancestry] [CEU] $r>0.67$) were used (rs7756992, rs10946398 and rs7754840). Type 2 diabetes risk variants within the *CDKAL1* locus were associated with reduced birthweight in a fixed-effect meta-analysis (per allele $\beta=-20$ g [95% CI −29, −11], $p=5\times10^{-6}$) (Fig. 1). Likewise, the *HHEX-IDE* rs1111875 type 2 diabetes risk allele was associated with reduced birthweight (per allele $\beta=-16$ g [95% CI −24, −8], $p=8\times10^{-5}$) (Fig. 2).

No other published type 2 diabetes variants were associated with reduced birthweight in our meta-analyses

Table 1 Linear regression analyses of fetal genotype and birthweight in 4,213 individuals from the Danish Inter99 study population

Type 2 diabetes risk		Mean birthweight (g)[a] per genotype (number of T2D risk alleles)			Effect	
Risk variant[b]	Allele frequency	0	1	2	Per T2D risk allele (g)[c]	*p* value[d]
Beta cell dysfunction						
KCNJ11 rs5219	0.37	3,502±459	3,478±441	3,498±449	−5 (−25, 15)	0.62
TCF7L2 rs7903146	0.27	3,495±453	3,481±441	3,487±453	−8 (−29, 13)	0.45
IGF2BP2 rs4402960	0.30	3,484±448	3,491±442	3,500±475	3 (−17, 24)	0.75
WFS1 rs10010131	0.58	3,470±478	3,491±453	3,502±432	19 (−1, 39)	0.062
SLC30A8 rs13266634	0.68	3,534±444	3,477±444	3,488±454	−14 (−34, 6)	0.17
CDC123 rs12779790	0.19	3,486±444	3,498±458	3,466±465	4 (−20, 28)	0.75
TSPAN8 rs7961581	0.27	3,483±442	3,494±455	3,487±472	13 (−9, 34)	0.25
KCNQ1 rs2237895	0.41	3,489±442	3,489±457	3,490±444	−6 (−25, 14)	0.59
MTNR1B rs10830963	0.27	3,486±448	3,488±447	3,511±466	9 (−13, 30)	0.43
GCK rs1799884	0.16	3,492±452	3,482±443	3,496±471	−9 (−35, 17)	0.50
DGKB rs2191349	0.51	3,467±442	3,491±443	3,498±462	13 (−6, 32)	0.17
PROX1 rs340874	0.54	3,491±429	3,498±455	3,470±453	−13 (−32, 6)	0.19
HHEX-IDE rs1111875	0.59	3,514±454	3,484±445	3,483±452	−17 (−36, 2)	0.088
CDKAL1 rs7756992	0.28	3,498±451	3,483±442	3,462±4586	−22 (−43, −1)	0.044
CDKN2A/2B rs10811661	0.83	3,476±418	3,498±460	3,486±445	−2 (−28, 24)	0.88
TCF2 rs7501939	0.41	3,495±447	3,482±452	3,498±443	−4 (−24, 15)	0.66
JAZF1 rs864745	0.51	3,465±448	3,505±449	3,482±452	8 (−11, 28)	0.39
Insulin resistance						
GCKR rs780094	0.65	3,460±438	3,499±450	3,486±451	7 (−13, 27)	0.50
PPARG rs1801282	0.87	3,421±401	3,486±453	3,492±450	12 (−16, 40)	0.41
IRS1 rs2943641	0.62	3,494±441	3,494±447	3,481±453	−8 (−27, 12)	0.43
ADAMTS9 rs4607103	0.78	3,519±442	3,474±457	3,495±445	6 (−17, 29)	0.62
Obesity						
FTO rs8050136	0.41	3,490±442	3,484±455	3,501±449	5 (−15, 24)	0.63
Unknown						
NOTCH rs10923931	0.10	3,485±447	3,505±457	3,439±414	9 (−23, 42)	0.57
THADA rs7578597	0.90	3,541±526	3,501±436	3,485±450	−23 (−54, 9)	0.15
ADCY5 rs11708067	0.76	3,543±438	3,500±452	3,476±450	−33 (−55, −10)	0.0040

[a] Means ± SD stratified by fetal type 2 diabetes risk genotypes

[b] Grouped according to their assumed phenotypical effect

[c] Effect in grams (95% CI)

[d] Bonferroni threshold for 25 test was $p<0.002$; effects and *p* values were calculated assuming an additive genetic model adjusted for sex, maternal diabetes status and parity

T2D, type 2 diabetes

(ESM Fig. 1). However, fetal *TCF7L2* rs7903146 and *CDKN2A/B* rs10811661 type 2 diabetes risk alleles were associated with slightly increased birthweight (ESM Fig. 1a, f). No heterogeneity was observed in any of the meta-analyses ($p>0.2$).

Combined effect of HHEX-IDE, CDKAL1 and ADCY5 type 2 diabetes risk alleles on birthweight Information from the *CDKAL1, HHEX-IDE* and *ADCY5* variants in the Danish population was combined into a fetal risk allele score (0–6 risk alleles) and the association with birthweight was tested. This analysis showed an average birthweight difference of −22 g [95% CI −34, −10], $p=0.0003$ per risk allele (Fig. 3). The 14% of the examined Inter99 population carrying five to six risk alleles weighed on average 110 g [95% CI 42, 179] less than the 6% carrying zero to one risk alleles. In combined analyses, the three variants were also associated with modest decreases in birth length and ponderal index

Table 2 Quantitative linear regression analyses of fetal genotype, and ponderal index and birth length in 4,213 individuals from the Danish Inter99 population

T2D risk		Value per genotype (number of T2D risk alleles)[a]			Effect[b]	
Risk variant	Allele frequency	0	1	2	Per T2D risk allele	*p* value[c]
Mean PI (kg/m^3)						
ADCY5 rs11708067	0.76	25.0±2.3	24.8±2.3	24.7±2.3	−0.1 (−0.2, −0.02)	0.024
HHEX rs1111875	0.59	24.9±2.3	24.8±2.2	24.8±2.3	−0.05 (−0.2, 0.04)	0.28
CDKAL1 rs7756992	0.28	24.8±2.3	24.8±2.3	24.7±2.3	−0.02 (−0.1, 0.09)	0.78
Mean BL (cm)						
ADCY5 rs11708067	0.76	52.1±1.8	52.0±1.9	52.0±1.9	−0.08 (−0.17 0.02)	0.12
HHEX rs1111875	0.59	52.1±2.0	52.0±1.9	52.0±1.9	−0.04 (−0.12, 0.04)	0.31
CDKAL1 rs7756992	0.28	52.0±1.9	52.0±1.9	51.9±1.9	−0.10 (−0.19, −0.01)	0.036

Data are stratified according to fetal type 2 diabetes risk genotypes of *ADCY5*, *HHEX* and *CDKAL1*

[a] In kg/m^3 for PI, in cm for BL, mean ± SD

[b] In kg/m^3 for PI, in cm for BL effect (95% CI)

[c] Bonferroni threshold for 6 test was $p<0.0083$; effects and *p* values were calculated assuming an additive genetic model adjusted for sex, maternal diabetes status and parity

BL, birth length; PI, ponderal index; T2D, type 2 diabetes

(per allele: β=−0.07 cm [95% CI −0.12, −0.01], p=0.01 and β=−0.07 kg/m^3 [95% CI −0.13, 0.00], p=0.04, respectively).

Combined analyses of effect of 25 type 2 diabetes risk alleles on birthweight To estimate the effect of carrying a high vs low load of risk alleles, a combined analysis of all 25 variants was performed. Information from the 25 type 2 diabetes risk variants was combined into a binary risk allele score (low vs high genetic risk) and plotted against birthweight (Fig. 4). The association with birthweight was tested. Individuals with a high risk allele score (≥25 type 2 diabetes risk alleles) were on average slightly lighter than individuals with a low risk allele score (<25 type 2 diabetes risk alleles) (β=−35 g [95% CI −69, −2], p=0.037). A similar analysis excluding the *HHEX-IDE*, *CDKAL1* and *ADCY5* variants failed to reveal a significant effect (β=−24 g [95% CI −56, 9], p=0.16).

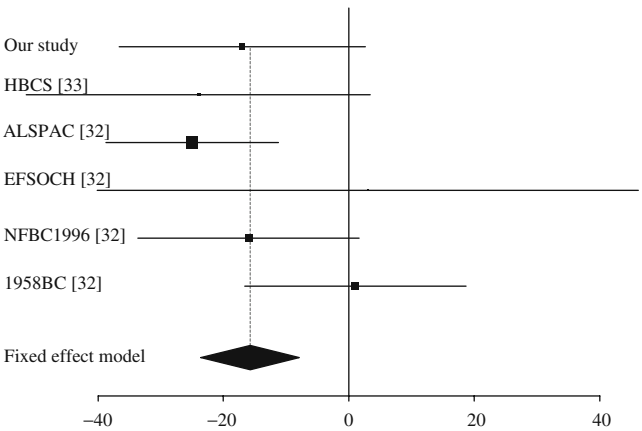

Fig. 1 Meta-analysis of *HHEX-IDE* rs1111875 fetal genotype and birthweight including up to 25,164 European individuals. Effect size estimates and standard errors obtained from previous published studies [32, 33] and the present study were combined in a meta-analysis using the inverse variance method. Black diamond, combined change in birthweight per fetal risk allele (β=−16 g [95% CI −24, −8], $p=8\times10^{-5}$); black squares, effects in single studies sized according to their weight in the meta-analysis

Discussion

Our analysis of the fetal genotype of 25 type 2 diabetes risk variants showed a novel association between *ADCY5* rs11708067 risk allele and birthweight, with a 33 g reduction in birthweight per risk allele. Although this novel finding does not withstand correction for multiple testing in the Inter99 study population, independent statistical evidence for this locus was provided by Freathy et al. at the ASHG meeting 2009 [39]. Moreover, in meta-analyses we confirmed that the risk-conferring alleles at *CDKAL1* and *HHEX-IDE* loci are associated with lower birthweight. Finally, we showed that no strong general effect on birthweight can be ascribed to the 25 type 2 diabetes risk alleles confirmed as of today. Our study thus adds important knowledge to current understanding of the effect of genes on birthweight and subsequent development of type 2 diabetes.

As fetal insulin is a crucial fetal growth factor, the ‘fetal insulin hypothesis’ suggests that genetic variants predis-

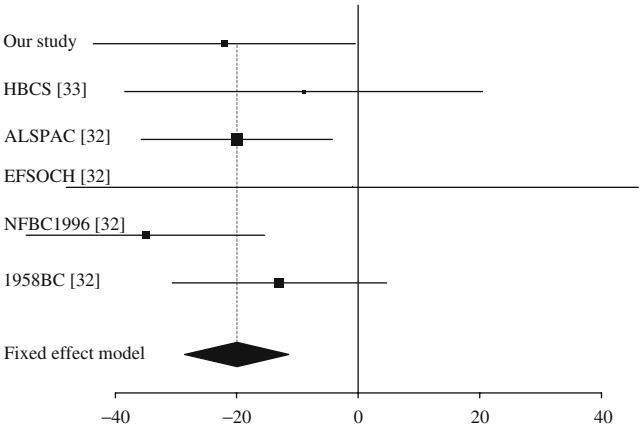

Fig. 2 Meta-analysis of *CDKAL1* fetal genotype and birthweight including 24,885 European individuals. Effect size estimates and standard errors obtained from previous published studies [32, 33] and the present study were combined in a meta-analysis using the inverse variance method. Three different proxies rs7756992 (present study), rs7754840 [33] and rs10946398 [32] in linkage disequilibrium (HapMap CEU $r>0.67$) were used. Black diamond, combined change in birthweight per fetal risk allele ($\beta=-20$ g [95% CI −29, −11], $p=5\times10^{-6}$); black squares, effects in single studies sized according to their weight in the meta-analysis

posing to decreased insulin secretion or action causes reduced intrauterine growth and thereby lower birthweight as well as late-onset type 2 diabetes [5]. This hypothesis assumes that insulin deficiency is already present during fetal life. Indeed, the birthweight-lowering alleles of *CDKAL1* and *HHEX-IDE* predispose to type 2 diabetes due to reduced insulin secretion and beta cell dysfunction [11, 40]. The associations with lower birthweight for these two loci therefore support the 'fetal insulin hypothesis' by

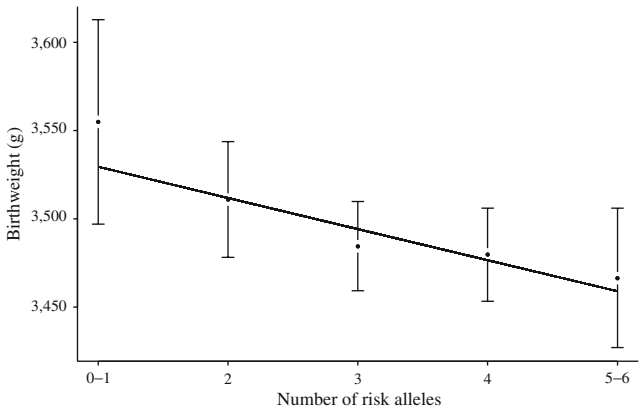

Fig. 3 The association between birthweight and the number of fetal type 2 diabetes risk alleles at *CDKAL1* (rs7756992), *HHEX-IDE* (rs1111875) and *ADCY5* (rs11708067) in the Danish Inter99 population (n=4,213). Raw birthweight data (mean, SD) were plotted according to the number of risk alleles. Analyses were performed by summing up risk alleles assuming an additive genetic model adjusted for sex, maternal diabetes status and parity. Effect per allele: $\beta=-22$ g (95% CI −34, −10), $p=3\times10^{-4}$

indicating that beta cell dysfunction may already be present in pre-natal life. This contrasts with other type 2 diabetes risk alleles that are also believed to increase susceptibility to type 2 diabetes through decreased beta cell function, but which were not found to have a decreasing effect on birthweight by us and others [32–34, 36]. No strong phenotype related to beta cell function has been reported for variants at the *ADCY5* locus [13]; therefore the mechanism by which the *ADCY5* risk allele decreases birthweight and increases the risk of type 2 diabetes may be different. Interestingly, the *ADCY5* variant, but not the *HHEX-IDE* or *CDKAL1* variant, was also significantly associated with a lower ponderal index, indicating that newborns with the *ADCY5* variant had disproportional intrauterine growth. Thinness at birth, as reflected by a lower ponderal index, has been related to insulin resistance and type 2 diabetes later in life [41]. The *CDKAL1* locus was significantly associated with reduced birth length, while *ADCY5* and *HHEX-IDE* loci were not. These heterogeneous associations for the three loci could indicate differential effects, but may also be due to study sample size and thereby to lack of statistical power.

The *ADCY5* locus was initially identified in a large meta-analysis of fasting plasma glucose levels, including data from 21 genome-wide association studies conducted by the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) investigators [13, 31]. The intronic rs11708067 variant was associated with increased fasting plasma glucose levels (0.027 mmol/l, $p=1.7\times10^{-14}$) and risk of type 2 diabetes (OR 1.12, $p=9.9\times10^{-21}$) at a genome-wide level [13]. In the same study, the rs11708067 risk allele was also associated with decreased HOMA of beta cell function (HOMA-B) ($p=3.6\times10^{-8}$) but not with HOMA of insulin resistance ($p=0.16$). Simultaneously, a large meta-analysis undertaken by MAGIC investigators on post-OGTT values identified the *ADCY5* locus to be associated with increased 2 h plasma glucose (0.09 mmol/l, $p=4.2\times10^{-16}$) [31]. This association was reported with another single nucleotide polymorphism (rs2877716) in strong linkage disequilibrium with rs11708067 (HapMap CEU population $r^2=0.82$). The rs2877716 variant was also associated with lower 2 h serum insulin levels adjusted for 2 h plasma glucose levels ($p=1.43\times10^{-6}$), but not with the insulinogenic index or AUC for insulin and glucose ($p>0.1$) [31]. As variation in *ADCY5* is associated with higher fasting and 2 h plasma glucose levels, as well as with lower HOMA-B and 2 h serum insulin levels in non-diabetic individuals, the *ADCY5* variants may predispose to type 2 diabetes and low birthweight through an effect on insulin secretion rather than through insulin resistance. During revision of this manuscript Freathy et al. reported a meta-analysis of genome-wide association studies followed by replication studies showing that the C allele of rs9883204

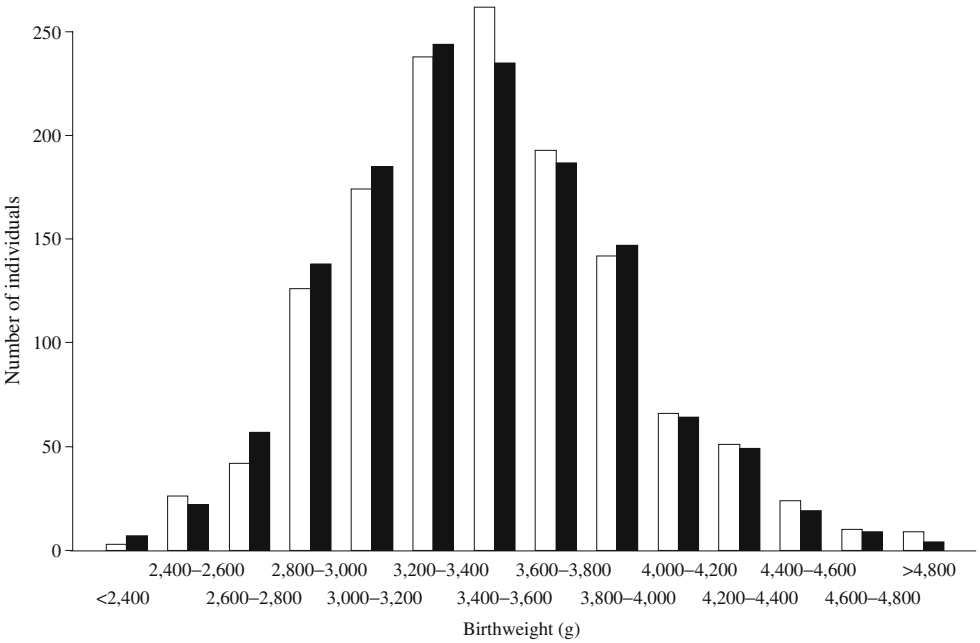

Fig. 4 Distribution of birthweight in 2,733 individuals with low (n=1,366; white bars) and high (black bars) genetic risk (n=1,367) from the Inter99 study. Raw birthweight data were plotted against number of individuals with either a low (<25 risk alleles) or high (≥25 risk alleles) genetic risk score. The effect, calculated adjusted for sex, maternal diabetes status and parity, was β=−35 g (95% CI −69, −2), p=0.037

in *ADCY5* in linkage disequilibrium with rs11708067 (HapMap CEU phase III r^2=0.72) was associated with lower birthweight ($p=7\times10^{-15}$) [42].

ADCY5 is expressed in multiple tissues including the pancreatic islets and beta cells, but with the highest expression levels observed in the heart and brain [13, 43]. *ADCY5* (also known as *AC5*) encodes adenylate cyclase 5 (ADCY5), which catalyses generation of cyclic AMP. ADCY5 may be involved in insulin release, since cyclic AMP mediates activation of protein kinase A, which induces calcium influx, subsequent insulin secretion and transcription of the proinsulin gene [44]. Interestingly, *Adcy5*-knockout mice live significantly longer than control mice [45]. This has been proposed to be due to a protective effect on the heart, where disruption of ADCY5 inhibits cardiac apoptosis and thereby protects against pressure overload and oxidative stress [45, 46]. From these observations, it could be speculated that ADCY5 may also be involved in apoptosis of beta cells, possibly leading to decreased beta cell mass and decreased insulin-secreting capacity. This scenario could explain the associations with type 2 diabetes and low birthweight.

In the Danish Inter99 population as such, we were only able to show significant associations for the *CDKAL1* and *ADCY5* loci. In the present meta-analyses, we confirmed the associations with decreased birthweight for the *HHEX-IDE* and *CDKAL1* loci. The lack of significant association for the *HHEX-IDE* variant in the Inter99 population may be due to insufficient statistical power, if the variant has a less pronounced impact on birthweight, as suggested previously [34]. In meta-analyses we also observed that fetal *TCF7L2* rs7903146 and *CDKN2A/B* rs10811661 risk alleles were associated with slightly increased birthweight. Mother–offspring pair analyses have previously shown that this effect of the *TCF7L2* risk allele is merely a reflection of the maternal genotype effect, which is observed because maternal and fetal genotypes are 50% correlated [36]. A maternal type 2 diabetes risk genotype may predispose to increased birthweight due to a predisposition towards increased glucose levels during pregnancy. The same explanation could account for the reported association between the *CDKN2A/B* risk allele and increased birthweight.

Although the individual effects of the three type 2 diabetes risk variants on birthweight are relatively small, combined additive analyses of *CDKAL1*, *HHEX* and *ADCY5* showed a mean birthweight reduction of 110 g for carriers of five or six risk alleles compared with carriers of zero or one risk alleles. This magnitude can be compared with the impact of mothers smoking four additional cigarettes per day during third trimester of pregnancy [47].

The combined analysis of all 25 type 2 diabetes risk variants showed that individuals belonging to the high-risk group weighed marginally less than individuals belonging to the low-risk group. No significant effect was observed when excluding *HHEX-IDE*, *CDKAL1* and *ADCY5* from the analysis. This indicates that no strong general effect on birthweight can be ascribed to these 25 risk alleles. Together with the single variant analyses, the results from the combined analyses suggest that for some of the variants insulin deficiency and/or resistance may only be present later in life, while for others the defect may already be noticeable in pre-natal life. However, concordance between maternal and fetal genotypes may potentially offset the impact of fetal risk alleles, because mothers of newborns with a high number of risk alleles are likely to have a high

number of risk alleles themselves, making them more susceptible to hyperglycaemia during pregnancy. Interestingly, none of the variants associated with insulin resistance seem to be associated with low birthweight. The 25 type 2 diabetes variants examined in the present study explain only ~2% of the total variation in birthweight. The heritability of birthweight is 38%, as estimated in a Danish population-based twin cohort [48], suggesting that multiple additional genetic variants are likely to be involved. These may include yet undiscovered low-frequency variants overlapping with variants involved in type 2 diabetes pathogenesis, as well as variants with no influence on type 2 diabetes development. Moreover, the heritability estimate also suggests that the variation in birthweight is largely explained by non-genetic factors that may affect the intrauterine environment and thereby fetal growth [4, 48].

To strengthen the analyses, we have in the present study of Inter99 participants adjusted the analyses for sex, maternal diabetes status and parity, since these variables all affect birthweight in this population (personal communication, K. Pilgaard, Steno Diabetes Center, Gentofte, Denmark). Maternal diabetes status is related to genotype and offspring birthweight, and is therefore considered a confounding factor. In this study parental diabetes status was assessed through a questionnaire and age of onset was not reported, which is a limitation. However, higher birthweight in newborns whose mothers develop diabetes at some point in adult life has been reported [49]. This observation is probably explained by maternal hyperglycaemia during pregnancy, masking genetic effects working in the opposite direction and making it important to adjust for this confounder. However, adjusting for maternal diabetes status assessed several years after the pregnancy may not sufficiently account for the total effect of gestational hyperglycaemia, so we may have underestimated the effect of type 2 diabetes gene variants on birthweight in this study.

Another limitation of this study is the lack of exact information regarding gestational age between weeks 37 and 42. However, none of the investigated variants have to our knowledge been associated with gestational age and thus an even genotype distribution can be presumed. In addition, we do not have information on parental genotypes and could not therefore exclude a parental genotype effect.

In conclusion, we report a novel association with decreased birthweight in carriers of the *ADCY5* type 2 diabetes risk allele. We also confirm associations with lower birthweight for the *HHEX-IDE* and *CDKAL1* type 2 diabetes risk alleles in large meta-analyses.

Acknowledgements The authors wish to thank A. Forman, I.-L. Wantzin and M. Stendal for technical assistance, and A. L. Nielsen, G. Lademann and M. M. H. Kristensen for management assistance. The study was supported by grants from the Lundbeck Foundation Centre of Applied Medical Genomics for Personalized Disease Prediction, Prevention and Care (LuCAMP), the Danish Health Research Council, the European Union (EXGENESIS, grant no. LSHM-CT-2004-005272), the Danish Diabetes Association, the Danish Council for Independent Research (Medical Sciences) and Novo Nordisk. The Inter99 was initiated by T. Jørgensen (principal investigator), K. Borch-Johnsen (co-principal investigator), H. Ibsen and T. F. Thomsen. The Steering Committee comprises the former two and C. Pisinger. The study was financially supported by research grants from the Danish Research Council, the Danish Centre for Health Technology Assessment, Novo Nordisk, Research Foundation of Copenhagen County, Ministry of Internal Affairs and Health, the Danish Heart Foundation, the Danish Pharmaceutical Association, the Augustinus Foundation, the Ib Henriksen Foundation, the Becket Foundation and the Danish Diabetes Association.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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