Genetic Determinants of Adiponectin Regulation Revealed by Pregnancy

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Abstract

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Objective—We investigated genetic determinants of adiponectin during pregnancy to reveal novel biology of adipocyte regulation.

Methods—We conducted a genome-wide association study in 1,322 pregnant women from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study with adiponectin measured at ~28 weeks of gestation. We selected variants reaching $P<5\times10^{-5}$ for *de novo* genotyping in two replication cohorts (Genetics of Glycemic regulation in Gestation and Growth (Gen3G) N=522; ECOGENE-21 N=174).

Results—In the combined meta-analysis, the maternal T allele of rs900400 located on chr3q25 (near *LEKR1/CCNL1*) was associated with lower maternal adiponectin (β±SE= −0.18±0.03 SD of adiponectin per risk allele; $P=1.5\times10^{-8}$; N=2004; multivariable adjusted models). In contrast, rs900400 showed only nominal association with adiponectin in a large sample of non-pregnant women (β±SE= −0.012±0.006; $P=0.05$; N=16,678 women from the ADIPOgen consortium). Offspring rs900400 T risk allele was associated with greater neonatal skin fold thickness (β ±SE=0.19±0.04 SD per risk allele; $P=4.1\times10^{-8}$; N=1489) and higher cord blood leptin (β ±SE=0.28±0.05 log-leptin per risk allele; $P=8.2\times10^{-9}$; N=502), but not with cord blood adiponectin ($P=0.23$; N=495). T allele of rs900400 was associated with higher expression of *TIPARP* in adipocytes.

Conclusion—Our investigations of adipokines during pregnancy and early life suggest that rs900400 has a role in adipocyte function.

Introduction

Adipose tissue is a key regulator of insulin sensitivity, partly through the endocrine functions of adipokines. Healthy 'metabolically flexible' adipose tissue is characterized by small adipocytes that secrete high levels of adiponectin, while large hypertrophic adipocytes in macrophage-infiltrated adipose tissue produce less adiponectin and high levels of leptin.[1] In human studies, low adiponectin levels are associated with lower insulin sensitivity and increased risk of type 2 diabetes (T2D) and gestational diabetes mellitus (GDM). [2, 3, 4] The most recent genome-wide association study (GWAS) of adiponectin levels identified 10 loci,[5] highlighting *ADIPOQ* as the strongest genetic determinant of adiponectin levels, confirming candidate gene investigations.[6] Despite GWAS[5, 7] and candidate gene[6] investigations of adiponectin, we know very little about the regulation of adipocytes' endocrine function. Investigating genetics of adipokines in the context of physiologic challenge could increase our understanding of adipose tissue 'flexibility'.

Pregnancy is characterized by major physiologic changes, including a marked decrease in insulin sensitivity. White adipose tissue expresses lower amounts of adiponectin in late gestation and levels decrease over the course of pregnancy.[8] Pregnancy may unmask metabolic risk, e.g. women with GDM are more likely to develop T2D.[9] We previously found genetic determinants of glycemic traits in pregnant women that were not identified in much larger studies of non-pregnant adults.[10] Given that, we hypothesized that pregnancy-

induced metabolic changes would enhance adipose tissue dysfunction in genetically predisposed women and allow detection of novel genetic determinants of adiponectin levels. Using an agnostic genome-wide discovery approach followed by replication, we investigated genetic determinants of adiponectin in 3 prospective cohorts of mother-newborn dyads. We pursued our main finding for associations with adiposity-related traits and other adipokines in mothers and newborns.

Methods

Description of participants

Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study – discovery GWAS—Detailed methods for recruitment and phenotyping of participants in the HAPO study were published previously.[11] In brief, pregnant women ≥ 18 years old were eligible if less than 32 weeks of gestation, had a singleton pregnancy, and had no history of diabetes. All women had a 75g oral glucose tolerance test (OGTT) between 24–32 weeks. All pregnant women gave written consent and an external Data Monitoring Committee provided oversight across sites. The original HAPO study enrolled women from diverse ancestry groups; main analyses for the present study included 1322 women of European ancestry who had consented to genetic studies and were included in a biomarkers sub-study in which adiponectin levels were measured. Newborns weight, length, and skin folds were measured within 72h of birth using standardized procedures.[12] Skin folds were measured in duplicate at three sites (flank, subscapular, and triceps) and summed; the average of two measurements at each site was used for analyses. Cord blood samples were collected at delivery, including circulating cells to obtain DNA. Only offspring whose mothers consented to genetic analyses are included in this analysis.

Adiponectin was measured using Luminex technology (Luminex Corp., Austin, TX) in stored (−80°C) maternal fasting samples collected at the time of the OGTT; the interassay coefficient of variation (CV; SD/mean) for low and high controls included with each assay was 11.3% and 15.1%, respectively.[13] DNA was prepared using the automated Autopure LS (Gentra Systems, Minneapolis, MN).

Genetics of Glycemic regulation in Gestation and Growth (Gen3G) cohort (replication)—Women planning to deliver at the Centre Hospitalier Universitaire de Sherbrooke (CHUS) were recruited between 6–15 weeks of pregnancy. Exclusion criteria were age <18 or >40 years old, multiple pregnancy, pre-gestational diabetes (type 1 or 2), diabetes discovered at 1st trimester, or medical conditions or medications that would affect glucose regulation. The CHUS ethical review board approved the project and all women provided written consent before inclusion in the study. This analysis includes up to 522 Gen3G women with adiponectin levels and genetic consent.

Details of Gen3G methods during pregnancy were published previously.[4, 14] Between 24–30 weeks of gestation, maternal anthropometry was measured using standardized procedure and each participant had a fasting 75g OGTT. At delivery, cord blood samples were collected, in addition to late pregnancy and peri-partum events from electronic medical records. Skin

folds were measured in duplicate at four sites (triceps, biceps, subscapular, and suprailiac) within 72h of delivery in a subsample, using standardized procedures.

Following collection, blood samples were maintained at 4°C and then centrifuged and stored at −80°C. Plasma glucose levels were measured by glucose hexokinase (Roche Diagnostics, Indianapolis, IN). Adiponectin was measured using radioimmunoassay (Millipore Corp, Billerica, MA). Leptin in maternal and cord blood was measured using Luminex technology (Human Milliplex, Millipore Corp, Billerica, MA). Intra- and inter-assay CVs were all<10%. DNA was extracted from maternal blood and from cord blood samples using the Gentra Puregene Cell Kit (Qiagene, Valencia, CA).

ECOGENE-21 Birth Cohort (replication)—Women with a singleton pregnancy in their 1st trimester were recruited from a founder population of French-Canadian origin (Saguenay area, Canada) and followed until delivery. Women over 40 years old and those with pre-gestational diabetes or other disorders known to affect glucose metabolism were excluded. The Chicoutimi Hospital Ethics Committee approved the project. All women provided written informed consent before inclusion in the study; 174 women who provided genetic consent were included in this analysis. Maternal anthropometric measurements were performed using standardized procedures. Glucose tolerance was assessed using a 75g OGTT performed at 24–30 weeks' gestation. Blood glucose levels were measured on fresh serum samples using a Beckman analyzer (model CX7; Fullerton, CA). Serum adiponectin levels were measured by ELISA (B-Bridge International). DNA was extracted from maternal blood samples using the Gentra Puregene Cell Kit (Qiagene, Valencia, CA). Newborns characteristics were collected at birth from clinical records.

Genotyping methods

Genome-wide genotyping of HAPO participants—DNA samples were genotyped using genome-wide arrays Illumina Human 610 Quad v1 B at the Broad Institute, as previously reported.[10] Genotype data that passed initial quality controls (QC) were released to the GENEVA Coordinating Center, National Center for Biotechnology Information database of Genotypes and Phenotypes (dbGaP), and HAPO study teams, who collectively performed QC using procedures previously described by the GENEVA consortium.[15] Poorly performing samples and SNPs were removed based on misspecified sex, chromosomal anomalies, unintended sample duplicates, sample relatedness, low call rate, high number of Mendelian errors, departures from Hardy-Weinberg equilibrium, duplicate discordance, sex differences in heterozygosity, and low minor allele frequencies, as detailed previously.[10, 16] Complete QC reports are available through dbGaP. http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000096.v4.p1

Genotyping Replication in Gen3G and ECOGENE-21 cohorts—Independent loci demonstrating an association with maternal adiponectin levels at $P<1 \times 10^{-5}$ in the HAPO GWAS (total 9 loci) were identified for replication in Gen3G maternal samples. One SNP (rs4943768) failed genotyping QC criteria and was excluded from meta-analyses. Among selected SNPs, the best candidate SNP with the lowest p-value after combining HAPO and Gen3G (rs900400) was further genotyped using the ECOGENE-21 maternal samples. In

Gen3G and ECOGENE-21 cohorts, selected SNPs were genotyped on a qRT-PCR (model 7500Fast, Applied Biosystems) using Applied Biosystems TaqMan probes and primers following the manufacturers' recommendations (Life Technologies Inc., Burlington, ON, Canada).

Statistical analyses

Genetic associations with maternal adiponectin—We used a z-score transformation for adiponectin in all cohorts. First, we performed a discovery GWAS of maternal adiponectin using SNPTEST v2 in 1322 HAPO women. We then performed a meta-analysis of HAPO and replication cohort(s): additive genetic linear regression models between maternal genotypes and maternal adiponectin levels (after z-score transformation) were adjusted for: Model 1: ancestry (for HAPO participants, using first two principle components), parity, maternal age, gestational age at OGTT, and neonatal sex; Model 2: Model 1 covariates and maternal mean arterial pressure, height and body mass index (BMI) at OGTT. Inverse variance-weighted meta-analysis results with $P<5.0 \times 10^{-8}$ were considered statistically significant.

Maternal rs900400 and adiposity/glycemia traits—To extend our understanding of our top finding on maternal adiposity and glucose regulation in pregnancy, we examined maternal rs900400 for association with maternal BMI, fasting, 1h and 2h glucose, and insulin sensitivity (z-score)[17] using meta-analysis across all three cohorts. Maternal leptin (log-transformed) was examined in Gen3G and adiponectin measured at both 1st and 2nd trimesters was evaluated in Gen3G and ECOGENE-21.

Offspring rs900400 and neonatal adiposity-related traits—Given prior reports of rs900400 association with birth weight and neonatal anthropometric measures,[18, 19] HAPO and Gen3G data were used to explore associations between offspring rs900400 genotype and neonatal adiposity-related traits not previously reported: cord blood C-peptide (z-score), adiponectin and leptin (only in Gen3G). We also confirmed associations with birth weight, birth length, ponderal index, and skin folds (z-score). Additive genetic models were adjusted for: Model 1: maternal age, parity, gestational age, and newborn sex; Model 2: Model 1 covariates and maternal BMI. $P<0.05$ was considered nominally significant; $P<0.007$ was considered statistically significant (Bonferroni corrected 0.05 divided by 7 neonatal traits). We conducted secondary analyses additionally adjusting for maternal genotype at rs900400.

Public data searches in non-pregnant individuals databases and functional data—To compare our findings in pregnant women with non-pregnant adults, genetic associations for rs900400 were explored using publicly available GWAS databases of anthropometric measures (Genetic Investigation of ANthropometric Traits (GIANT) consortium),[20, 21] glycemic-related traits (Meta-Analyses of Glucose and Insulin-related traits Consortium; MAGIC) and adiponectin levels (ADIPOgen). The potential function of rs900400 in adipocytes was examined by extracting eQTL with FDR q<0.01 from the publicly available Multiple Tissue Human Expression Resource (MuTHER).[22]

Results

Characteristics of HAPO, Gen3G, and ECOGENE-21 participants are presented in Table 1. All three cohorts were population-based with relatively similar characteristics: about half of the women were primiparous and mean mid-pregnancy BMI was in the overweight range. About half of newborns were male, and, by design, only term deliveries were included. We found weak correlations between maternal adiponectin levels and birth weight (HAPO r= −0.11; P<0.001; Gen3G r= −0.09; P=0.048).

Genetic associations with maternal adiponectin

The discovery GWAS in 1322 HAPO women revealed 9 independent loci associated with maternal adiponectin at 2nd trimester at P<1.0×10^{-5} (Figure S1) and we meta-analyzed 8 loci using HAPO and Gen3G data (Table 2). Meta-analysis of all three cohorts (N=2004 women) revealed that the maternal T allele at rs900400 located at chr3q25 (Figure 1a) was associated with lower 2nd trimester adiponectin ($\beta\pm SE$= −0.177±0.031 SD of adiponectin per risk allele; P=1.45×10^{-8} in Model 2; Table 3). The direction and effect sizes of maternal rs900400 T allele on adiponectin levels (SD per risk allele) were consistent in all 3 cohorts: $\beta\pm SE$= −0.189±0.038 in HAPO, −0.113±0.065 in Gen3G, and −0.252±0.104 in ECOGENE-21 (all Model 2). Secondary analyses including the fetal genotype in models slightly reduced the effect size, but the association remained strong in the same direction of effect (Model 2: $\beta\pm SE$= −0.141±0.038; P=2.5×10^{-4}; N=1261 mother-child pairs in HAPO). Maternal rs900400 seemed to have a smaller effect size for its association with adiponectin at 1st trimester (Model 2: $\beta\pm SE$= −0.118±0.064 SD of adiponectin per risk allele; P=0.07) vs. 2nd trimester (Model 2: $\beta\pm SE$= −0.166±0.065 SD of adiponectin per risk allele; P=0.01) in 498 women with levels measured at both time-points (Gen3G and ECOGENE-21), but the difference in effect sizes was not statistically significant (P=0.58). We did not find a significant association between maternal rs900400 and the change in adiponectin between 1st and 2nd trimester (Model 2: $\beta\pm SE$= −0.218±0.259 ug/mL adiponectin per risk allele; P=0.40).

At the *ADIPOQ* locus, the maternal G allele at rs17300539 (promoter region) was associated with lower adiponectin just below genome-wide significance ($\beta\pm SE$= −0.260±0.058 SD of adiponectin per risk allele; P=9.14×10^{-6} in Model 2; N=1842, Figure 1b). Another variant in *ADIPOQ* (rs17366568) showed suggestive association with adiponectin levels in pregnancy (P=2.01×10^{-5}), but other loci previously associated with adiponectin levels in non-pregnant population had weaker associations with maternal adiponectin levels in HAPO pregnant women compared to *ADIPOQ* variants (see Supplementary table 1).

Maternal rs900400 and adiposity/glycemia traits

The maternal adiponectin-lowering T allele at rs900400 was nominally associated with higher maternal glucose levels (fasting and 2h) and lower insulin sensitivity, but not maternal BMI (Table 3). We did not find associations between maternal rs900400 and maternal leptin during pregnancy (1st trimester $\beta\pm SE$= −0.003±0.037 log-leptin per risk allele; P=0.94; 2nd trimester $\beta\pm SE$= −0.010±0.035 log-leptin per risk allele; P=0.78; Model 2; N=505 Gen3G).

Offspring rs900400 and neonatal adiposity-related traits

In combined samples from HAPO and Gen3G, the offspring T allele at 900400 was associated with higher birth weight (β±SE=65.6±13.6 g per risk allele; P=1.51x10^{-6} in Model 2; N=1871) and sum of skin folds (β±SE =0.19±0.04 SD per risk allele; P=4.07x10^{-8} in Model 2; N=1489), confirming previous findings.[16, 18] Further adjustments for maternal genotype reduced effect sizes but associations remained statistically significant (Table 4). Genetic associations were modest for birth length and ponderal index (Table 4). In Gen3G, the offspring T allele was strongly associated with higher cord leptin (β±SE=0.277±0.047 log-leptin per risk allele; P=8.23x10^{-9}; N=502), while we observed no association with cord adiponectin (β±SE=0.449±0.371 SD of adiponectin per risk allele; P=0.23; N=495) (Figure 2).

Adiponectin and adiposity/glycemia-related traits in non-pregnant individuals (Table 5)

In ADIPOgen, the T allele was nominally associated with lower adiponectin in men (β±SE= −0.017±0.007 ln-adiponectin per risk allele; P=0.02; N=12,662) and women (β±SE= −0.012±0.006 ln-adiponectin per risk allele; P=0.05; N=16,678). In GIANT, we found no association with BMI (β±SE=0.005±0.004 kg/m^2 per risk allele; P=0.18; N=233,872) but the T allele was associated with adiposity distribution indices including waist-to-hip ratio (WHR) adjusted for BMI (β±SE=0.026±0.004 unit per risk allele; P=5.9x10^{-9}; N=141,215). This locus was identified as *LEKR1* in the latest GIANT GWAS[21] and the SNP reported (rs17451107) is in strong LD with rs900400 (r^2=0.932 in CEU). In MAGIC (up to 46,186 individuals), we found no association with fasting glucose (P=0.37) or fasting insulin (P=0.21).

Functional data in 3q25 region

We searched for eQTLs in the chr3q25 region in an adipose tissue expression dataset of publicly-available MuTHER database.[22] In this region, 789 SNPs were significantly associated (FDR   0.01) with the expression of 11 protein-coding. The T allele of rs900400 was associated with higher expression of *TIPARP* (P=6.75 x10^{-58}), but with no other transcript.

For potential functionality, we searched relevant ENCODE regulation tracks and findings from 3D chromatin contact partitions[23] for the chr3q25 region (Figure S2). According to 3D chromatin contact partitions defined in the GM12878 lymphoblastoid cell line using the DNA proximity ligation assay Hi-C, rs900400 and the promoters of the protein-coding genes *CCNL1, LEKR1, TIPARP,* and *SSR3* co-localize to the same genomic subcompartment of the A2 type, which is associated with high gene density, high expression and activating chromatin marks. While the GM12878 cell line is not representative of adipose tissue, chromatin contact is largely stable across cell lines.

Discussion

Our findings support that a genetic variant at 3q25 influences adipocyte function differently at diverse life stages. Starting from a genome-wide agnostic investigation, we demonstrated that the T allele at rs900400 is associated with lower adiponectin levels, specifically during

pregnancy. This is the same genetic variant for which the T allele in offspring was associated with higher birth weight in a prior meta-analysis from the EGG consortium[16, 19] and greater newborn adiposity in HAPO newborns.[16] It is also notable that rs900400 was previously associated with leptin levels ($\beta\pm$SE=0.030$\pm$0.005 log-leptin per risk allele; P=5.6x10^{-9} unadjusted for BMI; N=51,139 adults)[24] and with age at menarche ($\beta\pm$SE=0.03$\pm$0.005 year per risk allele; P=2.3x10^{-11}),[25] likely reflecting the role of adiposity in timing of puberty in women. Our current analyses revealed that the offspring T risk allele at rs900400 was strongly associated with higher cord blood leptin in newborns (P=8.23x10^{-9}; N=502). Therefore, the same risk allele is associated with varying phenotypes related to adipocyte function at different times over the life course.

Our findings support the concept that the association between rs900400 and adiponectin is enhanced by pregnancy-induced physiologic changes or that we have identified a genetic determinant of pregnancy-specific mechanisms of adiponectin regulation.[8] First, ADIPOgen data demonstrated only a modest association in a large sample of non-pregnant adults with similar effect sizes in men and women, arguing against a sex-specific effect. Second, the strength of association of adiponectin during pregnancy with maternal genotype at rs900400 compares favorably with rs17300539 in the promoter of *ADIPOQ*, the strongest genetic determinant of adiponectin in non-pregnant adults.[5, 6] Our observations suggest that pregnancy induces a 'metabolic stress test' on adipocyte function reflected by lower adiponectin, and indicate further a lack of adipose tissue flexibility in rs900400 risk allele carriers. On the other hand, our findings could also be interpreted as women carrying the T allele have a stronger physiologic response of pregnancy-related hypoadiponectinemia, a potential adaptive mechanism to deliver more nutrients to the fetus.[8] Adiponectin is exclusively produced by adipocytes, even in pregnancy,[26] in contrast to leptin, which is highly expressed by the placenta.[27] Pregnancy is characterized by an increase in multiple cytokines and hormones – estrogens, prolactin, cortisol, leptin – likely contributing to insulin resistance. Future functional studies may indicate whether some pregnancy-related cytokines/hormones mechanistically influence expression of adiponectin by interacting with rs900400.

Intriguingly, the risk variant for rs900400 in newborns demonstrated no association with cord adiponectin but did demonstrate strong association with cord leptin ($\beta\pm$SE=0.277$\pm$0.047 log-leptin per risk allele; P=8.23x10^{-9}; n=502). Previous GWAS of leptin levels in >50,000 adults had also revealed rs900400 as genetic determinant of leptin levels, but with a more modest effect size ($\beta\pm$SE=0.030$\pm$0.005 log-leptin per risk allele; P=5.6x10^{-9} unadjusted for BMI).[24] These observations are puzzling in the context of adipocyte biology. Newborn adiponectin levels are positively correlated with adiposity at birth, but inversely correlated with excess weight later in life. Leptin levels reflect overall adiposity in both adults and newborns. Adipocytes secrete a greater amount of leptin as they differentiate and grow larger, even when overfilled with triglycerides.[28] Small well-differentiated adipocytes produce high levels of adiponectin, but adiponectin secretion decreases as adipocytes become hypertrophic.[1] Given our findings, we hypothesize that rs900400 T allele carriers have adipocytes that allow greater fat accumulation within adipocytes, leading to higher adiposity and leptin levels at birth but to dysfunction of adipocytes and lower adiponectin in the face of specific 'environmental factors' such as

pregnancy-induced physiologic changes. In contrast to non-pregnant individuals[24], we did not find an association between maternal genotype and maternal leptin during pregnancy. This lack of association in our population of pregnant women could be related to the fact that circulating leptin during pregnancy is substantially derived from placental production, which might not be under the same genetic influence as adipose tissue.

Nominal associations of the maternal T risk allele rs900400 with greater insulin resistance and higher glycemia during pregnancy could be downstream effects of adipocyte function, either as an adaptive pregnancy-specific mechanism to deliver nutrients to the fetus or as a sign of adipose tissue maladaptation. On one hand, the T allele at rs900400 has been nominally associated with lower risk of T2D in the Nurses Health Study and Health Professionals Follow-up Study [29] suggesting a beneficial metabolic adaptation, yet this was not reported in larger GWAS.[30] On the other hand, associations with adiposity distribution indices in GIANT participants are in line with adipocyte dysfunction, as a lack of 'flexibility' in peripheral adipose tissue is believed to lead to central fat accumulation, represented by higher WHR. It is notable that we found absolutely no association of maternal rs900400 with BMI in our pregnant women nor in >233,000 GIANT participants, supporting the idea that this variant likely influences adipocyte *function* and is not an 'obesity' locus per se. In previous reports from GIANT, most WHR-loci were not associated with BMI and many genes at WHR-loci pointed to adipogenesis, embryonic development, and angiogenesis.[21]

In eQTL analyses, we found that the rs900400 T allele was associated with higher expression of *TIPARP* in adipocytes. *TIPARP* resides 374 Kb upstream from rs900400 and co-localizes to the same genomic subcompartment[23] (Figure S2). *TIPARP* suppresses glucose production, possibly by depleting NAD^+ levels, which may depress SIRT1 and ultimately PGC1α activity.[31] PolyADP-ribose polymerase (PARP) enzymes are emerging as coregulators of adipogenesis and glucose metabolism.[32] Among all tissues in the EBI Gene Expression Atlas, *TIPARP* is most highly expressed in adipose tissue,[33] and in GTEx pilot project data, *TIPARP* is highly expressed in visceral adipose tissue.[34]

Among loci that passed our initial discovery threshold but did not reach genome-wide significance after replication, we identified a few interesting biologic candidates, including *PPP1R3A, FOXO1,* and *FADS1. PPP1R3A* has been associated with rare severe insulin resistance disorders (with combined defect in *PPARG)*[35] and is part of the same family as *PPP1R3B,* which was recently associated with glycemic traits in pregnant women[10] and non-pregnant adults.[36, 37] *FOXO1* may regulate adipocyte differentiation[38] and mediate insulin action in adipose tissue and hepatocytes.[39] The *FADS1* locus has been associated with fasting glucose[36, 37] and multiple lipids and metabolites.[40] It is likely that our relatively small sample size limited our power to detect associations with adiponectin levels at these loci, but our observations suggest that pregnancy-induced physiologic changes enhance genetic associations with adipocyte function, lipids or insulin sensitivity pathways that otherwise necessitate much larger sample size to observe.

Our study was limited by sample size for some traits, and analyses were limited to women of European descent. Interpretation of gene expression microarray MUTHeR data is limited by

poor coverage of non-coding transcripts. Nevertheless, our study has numerous strengths. We tested genetic associations with many adiposity-related phenotypes in pregnant women and newborns using three population-based cohorts with prospective data/sample collection and standardized protocols. Moreover, we expanded our results by accessing publicly-available databases, including expression in adipocytes.

In conclusion, our findings suggest that rs900400 is implicated in adipocyte biology. T allele carriers at rs900400 have higher leptin levels and adiposity at birth, and female carriers show pregnancy-specific lowering of adiponectin levels. Investigating genotype-phenotype associations during pregnancy and early life permits the discovery of new biology not captured in genetic association studies conducted in general adult populations, and sheds new light on adipocyte endocrine function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is already known about this subject?

- Genetic determinants of adiponectin levels in general (non-pregnant) populations have been revealed by previous genome-wide association studies

- Some genetic determinants of glycemic-related traits have been found in pregnant women that were not identified in much larger studies of non-pregnant populations

- Pregnancy is characterized by major physiologic changes in insulin sensitivity and is considered a metabolic challenge on organs implicated in glycemic regulation, including adipose tissue

What does our study add?

- Starting from an agnostic genome-wide approach and followed by replication in cohorts of pregnant women, we revealed a novel genetic determinant of adiponectin (rs900400)

- This genetic variant (rs900400) in offspring was also strongly associated with leptin levels at birth, in line with associations with higher adiposity and birth weight previously reported

- Physiologic changes of pregnancy revealed genetic associations not otherwise detectable in general adult populations

Figure 1a
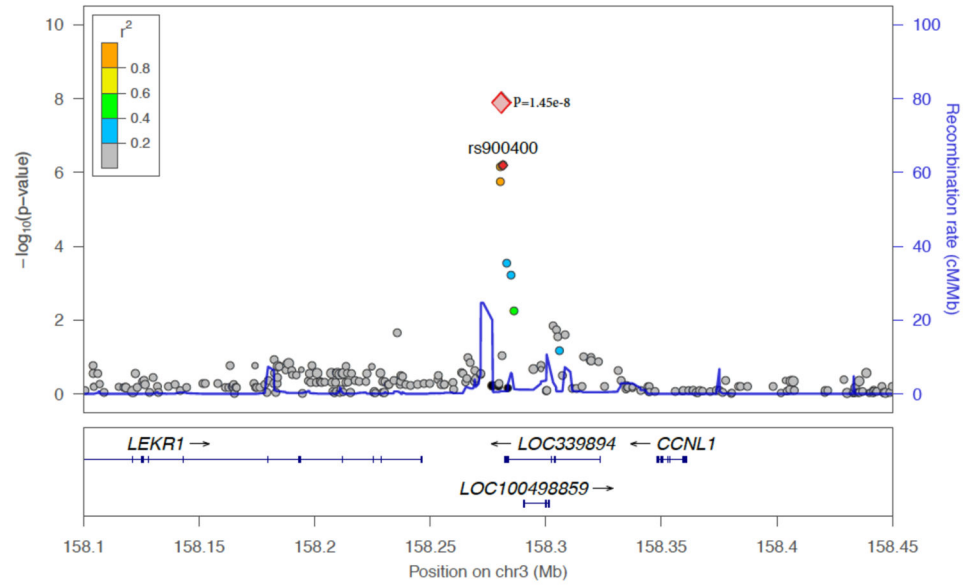
Figure 1b
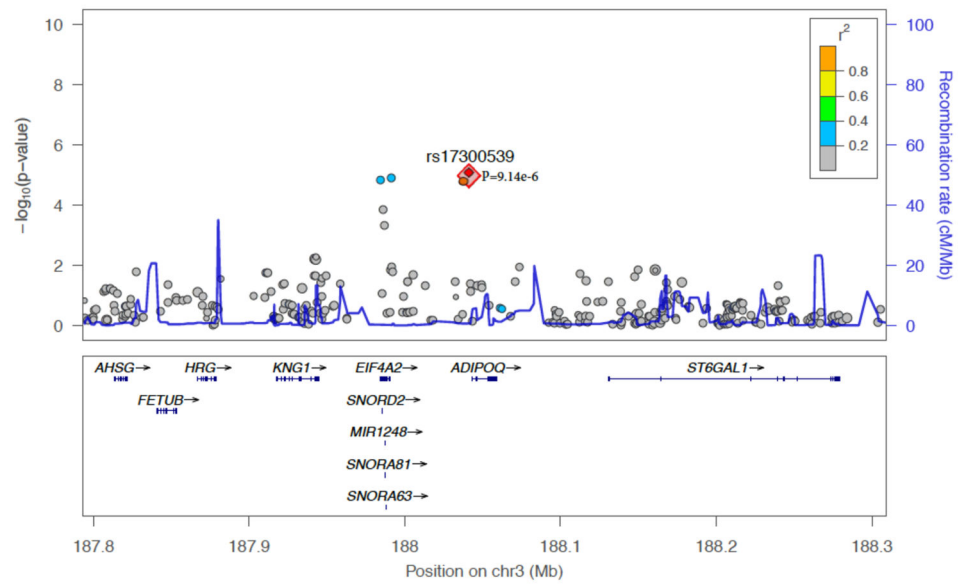
Figure 1.
Regional plots of 3q25 (Figure 1a) and of 3q27 (Figure 1b) for association with maternal adiponectin levels measured at 2nd trimester. Pink diamond indicated P-value of meta-analysis for fully adjusted Model 2.

Figure 2a   Figure 2b

Figure 2.
Cord blood leptin levels (Figure 2a; N=502) and adiponectin levels (Figure 2b; N=495) for each genotype at rs900400 in Gen3G newborns. Results presented for model 2, adjusted for maternal age, parity, gestational age at birth, newborn gender, and for maternal BMI

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Table 1

Maternal and neonatal characteristics of participants

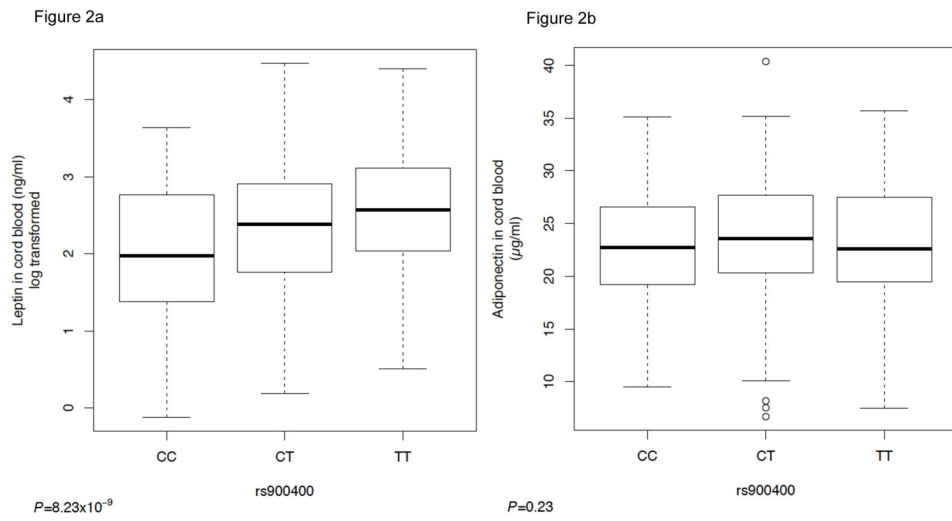|	HAPO n=1322	Gen3G n= 522	ECOGENE-21 n= 174
Maternal characteristics[a]	mean (SD)	mean (SD)	mean (SD)
Parity (% primiparous)	746 (56.4%)	261 (50.1%)	78 (44.8%)
Maternal age (years)	31.4 (5.3)	28.3 (4.3)	28.5 (3.8)
Gestational age at OGTT (weeks)	28.5 (1.4)	26.4 (1.0)	25.7 (1.1)
Body mass index (kg/m2)	28.4 (4.8)	28.1 (5.4)	27.6 (5.2)
Mean arterial pressure (mmHg)	83.8 (7.8)	81.3 (7.2)	81.7 (6.7)
Fasting adiponectin (ug/mL) [b]	19.9 (8.9)	12.5 (4.8)	10.5 (4.3)
Fasting leptin levels (ng/ml)	-	16.4 (10.4)	-
Fasting glucose (mmol/L)	4.56 (0.36)	4.20 (0.38)	4.36 (0.39)
1h-glucose (mmol/L)	7.30 (1.62)	7.10 (1.56)	7.75 (1.41)
2h-glucose (mmol/L)	6.06 (1.19)	5.78 (1.29)	6.68 (1.31)
Insulin Sensitivity index [b]	3.70 (1.49)	10.20 (5.86)	-
Neonatal characteristics	mean (SD)	mean (SD)	mean (SD)
Gender (% male)	661 (50.0%)	268 (51.3%)	96 (56.8%)
Gestational age at birth (weeks)	39.9 (1.2)	39.4 (1.3)	39.2 (1.5)
Birth weight (kg)	3.557 (0.518)	3.414 (0.462)	3.407 (0.463)
Birth length (cm)	51.8 (2.6)	50.9 (2.2)	49.8 (2.1)
Ponderal index (kg/m^3)	25.6 (3.2)	25.8 (2.5)	27.5 (2.6)
Sum of skin folds (mm) [c]	12.95 (2.63)	17.98 (3.33)	-
Cord blood C-peptide (ng/ml) [b]	1.05 (0.59)	0.47 (0.26)	-
Cord blood adiponectin (ug/mL)	-	23.3 (5.8)	-
Cord blood leptin levels (ng/ml)	-	14.9 (13.3)	-

[a]All maternal characteristics were measured at the time of OGTT, except for maternal age in Gen3G and ECOGENE-21 cohorts that was collected at first trimester.

[b]Absolute values differ because of bio-assays specific characteristics; all values z-score transformed before meta-analyses

[c]Sum of 3 folds in HAPO; sum of 4 folds in Gen3G; z-score transformed for analyses

Table 2

Results of meta-analyses of adiponectin levels in pregnant women in HAPO and Gen3G

SNP	Nearest gene	Chr	position	Effect allele	Effect allele frequency	N	Beta	SE	P-value	Beta	SE	P-value
							Model 1			**Model 2**		
rs900400	CCNL1	3	158281469	T	0.61	1830	−0.179	0.034	1.29E-07	−0.170	0.033	2.13E-07
rs17300539	ADIPOQ	3	188042154	G	0.92	1842	−0.266	0.060	1.05E-05	−0.260	0.058	9.14E-06
rs17171428	AMPH	7	38663569	T	0.98	1842	−0.457	0.118	1.12E-04	−0.421	0.114	2.23E-04
rs6958182	PPP1R3A	7	112945438	T	0.95	1836	−0.326	0.084	9.78E-05	−0.342	0.081	2.59E-05
rs6474834	NFIB	9	14426085	C	0.55	1839	−0.138	0.033	3.64E-05	−0.132	0.032	4.42E-05
rs198432	FADS1	11	61241557	C	0.75	1842	−0.132	0.038	4.89E-04	−0.122	0.037	8.50E-04
rs1408236	CSNK1A1L	13	36764897	A	0.98	1841	−0.604	0.144	2.82E-05	−0.644	0.141	4.77E-06
rs9934123	A2BP1	16	5723858	G	0.88	1838	−0.180	0.049	2.56E-04	−0.163	0.048	6.57E-04

Model 1: ancestry, parity, maternal age, gestational age at OGTT, and neonatal sex; Model 2: Model 1 covariates and maternal mean arterial pressure, height and body mass index (BMI) at OGTT.

Table 3

Association of maternal genotype T allele at rs900400 and maternal metabolic traits measured at 2nd trimester in meta-analyses of HAPO, Gen3G and ECOGENE-21 cohorts

Maternal metabolic traits at 2nd trimester	n	Model 1			Model 2		
		beta	SE	P-value	beta	SE	P-value
Adiponectin[a]	2004	−0.183	0.032	1.52×10^{-8}	−0.177	0.031	1.45×10^{-8}
Body mass index[b]	2059	0.001	0.003	0.62	-	-	-
Fasting glucose (mmol/L)	2060	0.033	0.012	0.005	0.029	0.011	0.009
1h glucose (mmol/L)	2052	0.055	0.051	0.28	0.044	0.050	0.38
2h glucose (mmol/L)	2061	0.086	0.039	0.03	0.076	0.038	0.046
Insulin Sensitivity[a]	1878	−0.087	0.034	0.01	−0.075	0.029	0.01

[a] z-score transformation before meta-analyses because of absolute units differences; betas express the change in SD of adiponectin per risk allele and the change in SD of log insulin sensitivity per risk allele.

[b] log transformed to achieve normality; betas express the change in log of BMI per risk allele.

Model 1: adjusted for ancestry, parity, maternal age, gestational week at the time of OGTT, and newborns' gender.

Model 2: Model 1 variables + maternal mean arterial blood pressure, maternal height and body mass index (all measured at the time of the OGTT; as presented in Table 1)

Table 4

Association of offspring genotype T allele at rs900400 and neonatal adiposity-related traits in meta-analyses of HAPO and Gen3G cohorts

Neonatal traits	n	Model 1			Model 2			Model 2 + maternal genotype		
		beta	SE	P-value	beta	SE	P-value	beta	SE	P-value
Birth weight (g)	1871	64.4	13.9	3.82×10^{-6}	65.6	13.6	1.51×10^{-6}	50.8	14.4	4.38×10^{-4}
Birth length (cm)	1868	0.210	0.064	0.001	0.213	0.064	0.001	0.145	0.067	0.03
Ponderal index (kg/m^3)	1868	0.194	0.092	0.04	0.199	0.091	0.03	0.182	0.091	0.06
Sum of skin folds [a]	1489	0.187	0.036	1.50×10^{-7}	0.192	0.035	4.07×10^{-8}	0.142	0.036	7.46×10^{-5}
Cord blood C-peptide [a]	1865	0.027	0.033	0.41	0.03	0.032	0.34	−0.021	0.034	0.53

[a] z-score transformation before meta-analyses because of absolute units differences; betas express the change in SD of sum of skin folds per risk allele and the change in SD of cord blood C-peptide per risk allele

Model 1: adjusted for maternal age, parity, gestational age at birth, and newborns' gender

Model 2: model 1 variables + maternal body mass index at the time of OGTT

Table 5

Look-ups for association of rs900400 with adiponectin levels, adiposity and glycemic traits in non-pregnant populations in publicly available databases

ADIPOgen consortium	http://www.mcgill.ca/genepi/adipogen-consortium							
	reference effect allele	other allele	effect allele frequency	beta	SE	P-value	N
Adiponectin [a] women	T	C	0.605636	−0.0118	0.0060	0.054	16678
Adiponectin [a] men	T	C	0.603885	−0.0166	0.0068	0.016	12662

GIANT consortium	http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files						
	reference effect allele	other allele	effect allele frequency	beta	SE	P-value	N
BMI all (EUR)	T	C	0.6083	0.005	0.004	0.18	233872
Waist women (EUR)	T	C	0.6083	0.013	0.0055	0.02	92566
Waist men (EUR)	T	C	0.6083	0.021	0.0066	0.001	61549
Waist all (EUR)	T	C	0.6083	0.016	0.0045	0.0003	153817
Waist adjBMI Women (EUR)	T	C	0.6083	0.029	0.0053	3.60E-08	91328
Waist adjBMI men (EUR)	T	C	0.6083	0.028	0.0066	2.10E-05	60800
Waist adjBMI all (EUR)	T	C	0.6083	0.029	0.0044	2.80E-11	151935
Waist-Hip ratio women (EUR)	T	C	0.6083	0.019	0.0054	0.0003	87485
Waist-Hip ratio men (EUR)	T	C	0.6083	0.031	0.0066	3.40E-06	57167
Waist-Hip ratio all (EUR)	T	C	0.6083	0.024	0.0043	3.80E-08	144465
Waist-Hip ratio adjBMI women (EUR)	T	C	0.6083	0.022	0.0054	5.40E-05	85508
Waist-Hip ratio adjBMI men (EUR)	T	C	0.6083	0.031	0.0068	4.20E-06	55843
Waist-Hip ratio adjBMI all (EUR)	T	C	0.6083	0.026	0.0044	5.90E-09	141215

MAGIC	http://www.magicinvestigators.org/downloads/						
	reference effect allele	other allele	effect allele frequency	beta	SE	P-value	N
Fasting glucose	t	c	0.603	−0.0035	0.004	0.37	~46,186
Fasting insulin	t	c	0.603	−0.0052	0.004	0.21	~46,186

[a] natural log-transformed adiponectin levels