Association of Adiponectin Gene Polymorphism With Birth Weight in Korean Neonates

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Adiponectin has been associated with insulin resistance and type 2 diabetes mellitus and possibly fetal growth. Our aim was to assess the association between the single nucleotide polymorphisms (SNPs) of the adiponectin gene (ADIPOQ) and the birth sizes. We investigated four SNPs of ADIPOQ (rs182052, rs2241766, rs1501299, and rs266729) and birth height and weight in 237 healthy full-term neonates. The neonates with the rs182052 G allele had a greater birth weight (p = 0.043 in the dominant model) and a higher ponderal index (p = 0.028 in the additive model). The rs2241766 G allele was associated with a greater birth weight (p = 0.016 in the recessive model). In a logistic regression analysis, the homozygotes for the rs182052 G allele and those for the rs2241766 G allele showed a significant association with a greater birth weight above 90 percentile (OR 2.75, 95% CI 1.13–6.70 and OR 5.15, 95% CI 1.66–15.99, respectively). In conclusion, we found an association between rs182052 and rs2241766 and birth weight and ponderal index among healthy neonates and suggested that adiponectin might have some roles in fetal growth.

Keywords: adiponectin, single nucleotide polymorphism, birth weight

Adiponectin is a 30-kDa protein secreted from adipocytes, and its serum levels are decreased in individuals with obesity, type 2 diabetes mellitus (T2DM), and conditions commonly associated with insulin resistance (Rasouli & Kern, 2008). Low serum adiponectin concentrations are also found in subjects with hypertension, atherosclerosis, coronary artery disease, or ischemic stroke (Lu et al., 2008; Rasouli & Kern, 2008). It has been suggested that adiponectin plays a role in the regulation of insulin sensitivity and glucose and lipid metabolism, and further in the development of insulin resistance, T2DM, and atherosclerosis (Rasouli & Kern, 2008; Yamauchi & Kadowaki, 2008). Not only does thiazolidinedione, an insulin sensitizer, increase circulating adiponectin level in humans, but administration of recombinant adiponectin improves insulin sensitivity in mice (Lu et al., 2008; Rasouli & Kern, 2008).

Adiponectin has also been suggested to have an important role in fetal growth. Cord blood adiponectin level was significantly higher than that of adults or adolescents. And, unlike with adults, cord adiponectin level was positively associated with birth size in many studies (Kadowaki et al., 2006; Kotani et al., 2004; Mantzoros et al., 2009). Cord blood adiponectin level was lower among small for gestational age (Kamoda et al., 2004). The key endocrine regulators on fetal growth are insulin and insulin-like growth factors (IGFs), and adiponectin has been known to have an effect on them as an insulin sensitizer (Briana & Malamitsi-Puchner, 2010; Inami et al., 2007; Mantzoros et al., 2004). Moreover, adiponectin in fetal life may have important implications for later health. Fetal growth and insulin resistance could affect growth and development of insulin resistance with metabolic consequences in later life (Nathanielsz, 1999).

Adiponectin concentrations are under strong genetic control with heritability between 30% and 50% (Siitonen et al., 2011). Some adiponectin gene (ADIPOQ) polymorphisms are reported to be associated with conditions such as insulin resistance or T2DM through serum adiponectin concentrations.
level (Melistas et al., 2009; Siitonen et al., 2011) and there may be an effect on fetal growth, although research is sparse. Therefore, we set out to investigate the association between the common single nucleotide polymorphisms (SNPs) of the ADIPOQ and birth sizes in South Korea.

Materials and Methods
Study Population and Data Collection Procedures
The study subjects were a total of 237 healthy full-term neonates born as singletons at Ewha Womans University Hospital, Seoul, South Korea, between July 2006 and December 2008. Their mothers were recruited among women who had received prenatal care at the hospital and agreed to participate in this study. Informed consent was obtained from the mothers. Information on maternal age and parity was obtained from a self-reported questionnaire completed during the first prenatal visit. We acquired information on infant sex, birth weight, birth length, and gestational age from medical records. Gestational age was determined according to the date of onset of the mother’s last menstrual period and by the ultrasonographic estimation. Ponderal index (kg/m^3) was calculated as the weight (kg) divided by height cubed (m^3). Neonates whose mother had hypertension, diabetes mellitus, or other diseases causing fetal growth restriction, or had received medications related to fetal growth, and those with congenital anomalies were excluded. Cord blood samples were taken immediately after delivery and aliquoted and stored at -70 degree Celsius until assayed. This study was approved by the institutional review board on human subjects at Ewha Womans University, Seoul, South Korea.

Genotyping Analysis
We selected four SNPs of ADIPOQ, which have not only been reported to be associated with adiponectin level and metabolic consequences such as diabetes and metabolic syndrome but also to be polymorphic, with the minor allele frequencies found in more than 20% of the Korean population. After DNA was extracted from whole blood using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), these genotypes of ADIPOQ (rs182052, rs2241766, rs1501299, and rs266729) were screened using the TaqMan fluorogenic 5′ nuclease assay (ABI, Foster City, CA, USA) and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA).

Statistical Analysis
The consistency of the genotype frequencies of each polymorphism with the Hardy–Weinberg equilibrium was assessed with the χ² test. The results are expressed as mean and standard deviation for continuous variables and as frequency and percentage for categorical variables.

The general associations between each polymorphism and birth size were assessed using general linear models with adjustment for sex, gestational age, and birth order. We further tested these associations under dominant, recessive, and additive genetic models only if the general association test was significant. The dominant genetic model compares neonates with one or more polymorphic alleles to those with homozygous wild-type alleles (Aa and AA vs. aa). The recessive genetic model compares neonates with a homozygous variant allele to those with one or more wild-type allele (AA vs. Aa or aa). The additive genetic model assumes that there is a linear gradient in birth size among three genotypes. Multiple tests for three genetic models of each SNP were adjusted by the Bonferroni correction (p value multiplied by the number of models).

In addition, we categorized neonates into three groups according to sex-specific percentile (<10 percentile as smaller birth size, >90 percentile as larger birth size, and 10–90 percentile) for birth weight, birth length, and ponderal index at birth. The cut points (10th and 90th percentiles) of height were 49.0 and 53.0 cm among boys and 48.0 and 52.0 cm among girls. Those of birth weight were 2,940 and 3,840 g among boys and 2,750 and 3,730 g among girls. Those of ponderal index were 24.0 and 29.4 kg/m^3 among boys and 24.2 and 29.8 kg/m^3 among girls. Multinomial logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of the risk associated with the variant allele for the smaller or larger birth sizes (height, weight, and ponderal index) in dominant and recessive models of each ADIPOQ polymorphism.

Pairwise linkage disequilibrium between each SNP was estimated using Haplovie (Barrett et al., 2005). Haplotype reconstruction analysis was done using PHASE (Stephens et al., 2001). We also performed general linear model and multinomial logistic regression analyses on haplotypes. We adjusted for sex, gestational age, and birth order of neonates as covariates in all analyses. All statistical analyses were performed using the SAS package (version 9.2, SAS institute, Cary, NC).

Results
The genotype distributions of the four SNPs and the success rates for genotyping are described in Table 1. The genotype distributions did not differ from those expected for a
population in the Hardy–Weinberg equilibrium using the \( \chi^2 \) test. There was significant linkage disequilibrium between the rs266729 and rs182052 \( (D’ = 1, R^2 = 0.349) \) and between rs2241766 and rs1501299 polymorphisms in the population \( (D’ = 1, R^2 = 0.183) \). The main haplotypes for rs266729/rs182052 were the C/G (53.6%), G/A (23.2%), and G/A (23.2%) types, and those for rs2241766/rs1501299 were the T/G (39.8%), G/G (32.4%), and T/T (27.8%) types in the population. Among subjects, homozygous diplotypes of C/A from the rs266729/rs182052 haplotypes (CA/CA) were 6.8%, heterozygous diplotype (CA/X) 29.5%, and non-T/G diplotypes (X/X) were 15.6%, 43.5%, and 41.4%, respectively.

The characteristics of the subjects are presented in Table 2. Among 237 neonates, 114 were boys (48.1%) and 123 were girl (51.9%). Forty-three percent were first born and 29% were born with cesarean section. Their gestational age ranged from 37.1 to 41.6 weeks, with a mean of 39.4 weeks. The mean and standard deviation values of birth height and weight were 49.9 ± 1.6 cm and 3,291 ± 351 g. There were four neonates with birth weight greater than 4 kg and no low birth weight infant (below 2.5 kg).

The association between each SNP and birth sizes is shown in Table 3. Two adiponectin SNPs (rs182052 and rs2241766) were significantly associated with birth weight and ponderal index at birth. The neonates homozygous for the common G allele of rs182052 had a greater birth weight (GG: 3,393 ± 387 g, GA: 3,241 ± 327 g, AA: 3,278 ± 336 g, \( p = .043 \) in the dominant genetic model) and a higher ponderal index (GG: 27.1 ± 2.6 kg/m\(^3\), GA: 26.5 ± 2.0 kg/m\(^3\), AA: 26.0 ± 1.9 kg/m\(^3\), \( p = .028 \) in the additive model) than those with the A allele. The variant G allele of rs2241766 was also associated with greater birth weight (TT: 3,257 ± 359 g, TG: 3,294 ± 317 g, GG: 3,513 ± 421 g, \( p = .016 \) in the recessive model and \( p = .059 \) in the additive model). No adiponectin SNPs in this study were significantly associated with birth height.

In the multinomial logistic regression analysis, the variant A allele of ADIPOQ rs182052 showed a significantly lower odds ratio for greater birth weight above 90 percentile (Table 4; OR 0.36, 95% CI 0.15–0.89 in the dominant genetic model) and a borderline significantly lower odds ratio for the higher ponderal index above 90 percentile (OR 0.42, 95% CI 0.17–1.01, \( p = .052 \) in the dominant model) relative to the common G allele. In other words, the neonates homozygous for the common G allele of rs182052 had a significant association with greater birth weight above 90 percentile \( (OR 2.75, 95\% CI 1.35–6.70) \) and a borderline significant association with a higher ponderal index above 90 percentile \( (OR 2.40, 95\% CI 0.99–5.79) \), compared with those with the GA or AA genotype. The homozygotes for the variant G allele of rs2241766 were significantly associated with greater birth weight above 90 percentile \( (OR 5.15, 95\% CI 1.66–15.99) \) in the recessive model.

In the haplotype analysis, the C/A haplotype from rs266729/rs182052 haplotypes was significantly associated

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**TABLE 1**

<table>
<thead>
<tr>
<th>SNP rs number (position)</th>
<th>Location</th>
<th>Genotyping success rate</th>
<th>Genotype</th>
<th>N (%)</th>
<th>Major allele frequency</th>
<th>H.W. p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs182052 (-10,066)</td>
<td>Intron 1</td>
<td>98.3%</td>
<td>GG</td>
<td>68 (29.2)</td>
<td>0.528</td>
<td>.43</td>
</tr>
<tr>
<td>rs2241766 (+45)</td>
<td>Exon 1</td>
<td>94.5%</td>
<td>TT</td>
<td>100 (44.6)</td>
<td>0.679</td>
<td>.35</td>
</tr>
<tr>
<td>rs1501299 (+276)</td>
<td>Intron 2</td>
<td>96.2%</td>
<td>GG</td>
<td>123 (53.9)</td>
<td>0.728</td>
<td>.47</td>
</tr>
<tr>
<td>rs266729 (-11,377)</td>
<td>Promoter</td>
<td>98.3%</td>
<td>CC</td>
<td>133 (57.1)</td>
<td>0.758</td>
<td>.80</td>
</tr>
</tbody>
</table>

Note: SNP = single nucleotide polymorphism; Position = position relative to ATG start; H.W. = Hardy–Weinberg equilibrium.

**TABLE 2**

<table>
<thead>
<tr>
<th>Baseline Characteristics of Neonates in the Study (n = 237)</th>
<th>N (%)</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>114 (48.1)</td>
<td>123 (51.9)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>123 (51.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First born</td>
<td>102 (43.0)</td>
<td>135 (57.0)</td>
<td></td>
</tr>
<tr>
<td>Second or greater born</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>168 (70.9)</td>
<td>69 (29.1)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation (week)</td>
<td>39.4 (1.0)</td>
<td>37.1–41.6</td>
<td></td>
</tr>
<tr>
<td>Height at birth (cm)</td>
<td>49.9 (1.6)</td>
<td>46.0–54.0</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3,291 (351)</td>
<td>2,520–4,240</td>
<td></td>
</tr>
<tr>
<td>Macrosomia (&gt;4 kg)</td>
<td>4 (1.7)</td>
<td>26.5 (2.2)</td>
<td>21.0–39.0</td>
</tr>
</tbody>
</table>

Note: Note: SD = standard deviation.
with birth weight. The neonates with the C/A haplotype had a lower birth weight (C/A carrier: 3,225 ± 305 g, non-C/A carrier: 3,328 ± 371 g, \( p = .048 \)) and a lower ponderal index (C/A carrier: 26.0 ± 2.0 kg/m², non-C/A carrier: 26.8 ± 2.2 kg/m², \( p = .014 \)). The presence of the C/A haplotype showed a significantly lower odds ratio for greater birth weight above 90 percentile (OR 0.26, 95% CI 0.07–0.92) relative to the absence of the C/A haplotype. The other haplotypes were not significantly associated with birth sizes.

### Discussion

In this study, we suggested that ADIPOQ SNP rs182052 and rs2241766 were probably associated with birth weight and
ponderal index at birth. Neonates with the SNP rs182052 common G allele had greater birth weight and higher ponderal index and rs2241766 variant G allele had greater birth weight. This is the first report that showed a significant association of ADIPOQ SNP rs182052 and rs2241766 of neonates with birth sizes.

Since the first report about an association of the ADIPOQ SNP rs182052 and serum adiponectin level by Woo et al. (2006), the variant A allele of this SNP has been shown to be associated with lower serum adiponectin level, insulin resistance syndrome-related phenotypes such as higher BMI, thicker skin fold thickness, higher fasting insulin, lower homeostasis model assessment of insulin sensitivity, and diabetic nephropathy (Bostrom et al., 2009; Ong et al., 2010; Richardson et al., 2006; Wassel et al., 2010). The association of ADIPOQ rs182052 with birth sizes was first reported in this study. The SNP rs182052 lies in the first intron of human adiponectin gene. Although introns are the non-protein-coding regions within a gene, they have recently been demonstrated to have an effect on messenger RNA (mRNA) degradation or translation suppression (Wassel et al., 2010). Also, this SNP might be involved in any change in a binding site of the transcription factor. The first intron of ADIPOQ contains a gene expression enhancer element, which CCAAT/enhancer-binding proteins (C/EBPα), a major transcription factor for adiponectin, bind to and could increase the activity of the adiponectin promoter (Ong et al., 2010; Qiao et al., 2005).

The reports about the association between the ADIPOQ rs2241766 and serum adiponectin level are more inconsistent. Some report a higher adiponectin level in the variant G allele (Jang et al., 2008; Siitonen et al., 2011) and others report no association between them (Chung et al., 2009; Kim et al., 2008). ADIPOQ rs2241766 is associated with T2DM, coronary artery disease, or carotid artery plaque in T2DM (Al-Daghri et al., 2011; Kim et al., 2008; Siitonen et al., 2011). The rs2241766 is located at exon 2 and is one of the most common polymorphisms of ADIPOQ. However, it causes synonymous mutation (GGT→GGG, Gly→Gly), that is, it does not make any change in amino acid sequences in adiponectin. So, the associations with some diseases or biologic functions were often explained by the possible linkage disequilibrium with other functional SNPs (Al-Daghri et al., 2011; Melistas et al., 2009). It is also suggested that this silent mutation may affect ADIPOQ expression by modifying mRNA splicing or stability (Al-Daghri et al., 2011; Melistas et al., 2009; Yang et al., 2003). Yang et al. (2003) showed the difference in allele-specific expression, with higher mRNA level transcribed by the G allele and lower level by the T allele of this SNP in the adipose tissue of heterozygous subjects. However, it is still not clear how this polymorphism could be associated with adiponectin level and other phenotypic variants or diseases.

The associations with genotypic variations of adiponectin and birth sizes have not been studied enough so far. As far as we know, there are only two studies that examined these genetic links. A study in Brazil showed that the variant A allele of SNP rs17300539 (-11,391) in the promoter region of ADIPOQ was associated with being born large for gestational age and with higher adiponectin levels at the age of 23–25 years (Bueno et al., 2010). Saïto et al. (2012) reported that the neonates with the variant G allele of SNP rs266729 (-11,377) in the vicinity of rs17300539 in the ADIPOQ promoter region had significantly greater birth weights and higher cord adiponectin levels. They suggested that the effect of the SNP rs266729 on birth weight is based on its relation to adiponectin level. In our study, the C/A haplotype from rs266729/rs182052 was associated with birth sizes, but this was similar to the rs182052 A allele, and the SNP rs266729 in the promoter region was not associated with birth sizes. The ADIPOQ SNP rs1501299 is also not associated with birth sizes in spite of the significant result of SNP rs2241766. The haplotypes, including SNPs rs1501299 and rs2241766, are associated with insulin resistance, metabolic syndrome, and T2DM (Chung et al., 2009; Jang et al., 2008; Melistas et al., 2009), but other studies have showed different associations with diseases between these two SNPs, similar to our study (Al-Daghri et al., 2011; Kim et al., 2008; Siitonen et al., 2011). These discrepancies in studies might be attributed to the variation of the linkage disequilibrium structures among the populations (Al-Daghri et al., 2011).

Although we did not have cord adiponectin level, it is reasonable to speculate that the effects of ADIPOQ polymorphisms on birth sizes were mediated by adiponectin level. The neonates with ADIPOQ SNP rs182052 G allele or rs2241766 G allele were likely to be associated with increase in birth weight and ponderal index through the higher concentration of cord adiponectin.

Among adults, the circulating adiponectin level decreases with obesity (Kotani et al., 2004; Rasouli & Kern, 2008). But blood adiponectin levels of neonates are two or three times higher than those of adults and were positively associated with birth weight and fat mass (Inami et al., 2007; Kadowaki et al., 2006; Kadowaki et al., 2006; Mantzoros et al., 2009; Saïto et al., 2012). This may be caused by a lack of negative feedback on adiponectin production, related to the relatively smaller fat mass, low proportion of visceral fat, different cell population (Briana & Malamitsi-Puchner, 2010; Kotani et al., 2004; Saïto et al., 2012), and multiple fetal tissues producing adiponectin (Briana & Malamitsi-Puchner, 2010; Kadowaki et al., 2006; Kotani et al., 2004). Higher adiponectin concentration among neonates than among adults, and lower adiponectin level in gestational diabetic mothers and their newborns suggests the important role of adiponectin in intrauterine growth and development and energy metabolism (Kiess et al., 2008). The important endocrine regulators on fetal growth are insulin
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and IGFs (Inami et al., 2007; Mantzoros et al., 2004) and adiponectin could be speculated to have a regulatory effect on fetal growth through enhancing insulin and IGF sensitivity and modulating their actions (Briana & Malamitsi-Puchner, 2010; Inami et al., 2007; Kadowaki et al., 2006; Kotani et al., 2004; Mantzoros et al., 2004). It also suggests that lower cord adiponectin concentration could be associated with weight gain in early childhood and a predisposition to insulin resistance or other metabolic consequences in later life (Kamoda et al., 2004; Mantzoros et al., 2009; Yang, 2009). However, the relationship between cord adiponectin level, birth sizes, and insulin sensitivity is still not clear (Briana & Malamitsi-Puchner, 2010; Jaquet et al., 2006; Kotani et al., 2004; Wang et al., 2010) and the cord adiponectin level was even considered as the mere reflection of fetal growth itself. Recently, it has been suggested that the low adiponectin level among obese adults is not a cause, but a consequence of obesity and adipose tissue-specific insulin resistance, and mediates insulin resistance in peripheral tissues and metabolic sequences (Lu et al., 2008; Yang, 2009). The links between birth weight, insulin resistance, and adiponectin need further delineation, and the genetic association between adiponectin polymorphisms and fetal growth also needs more study.

The limitation of our study is that we did not have cord adiponectin level and other serum biomarkers to explain the mechanism of the association between birth sizes and ADIPOQ polymorphism. Four SNPs in our study could not sufficiently cover the entire adiponectin gene so we might have missed some important regions and their effects. Also, we applied the correction for multiple comparisons across the genetic models but not across SNPs. In consideration of low replication in genetic association, we could only suggest but not confirm these associations in our study results and call for the replication studies. Nevertheless, we could show the probable association between ADIPOQ SNP rs182052 and rs2241766 and birth weight and ponderal index at birth among healthy neonates with a small proportion of macrosomia and no low birth weight babies, no neonates with mothers who had diabetes or other diseases, and those with congenital anomaly or neonatal complications as we wanted to know the effect on birth sizes itself among healthy neonates rather than on other diseases or conditions affecting birth sizes.

In conclusion, we found the association between the ADIPOQ SNP rs182052 common G allele and the rs2241766 variant G allele and greater birth weight and higher ponderal index at birth among healthy neonates for the first time and suggest that adiponectin might have some role in fetal growth.

Acknowledgments

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education Science and Technology (2011-0004200) and the Ewha Global Top5 Grant (2013) of Ewha Womans University.

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