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Original Article

Do Biochemical Markers and Apa I Polymorphism in IGF-II Gene Play a Role in the Association of Birth Weight and Later BMI?

Junqing WU [1, 2], Jingchao REN [1, 2], Yuyan LI [1,2], Yinjie WU [3], Ersheng GAO [1]

1. *Dept. of Epidemiology and Social Science on Reproductive Health, Shanghai Institute of Planned Parenthood Research/WHO Collaborating Center on Human Reproductive Health, Shanghai, China*
2. *Dept. of Epidemiology and Biostatistics, Fudan University, Shanghai, China*
3. *Jiangyin Maternal and Child Center, Wuxi, Jiangsu, China*

***Corresponding Author:** Tel: 86-021-64438402 Email: wujq1688@163.com

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Abstract

Background: The aim of the study was to explore the mechanisms underlying the association of birth weight with later body mass index (BMI) from the biochemical markers related to metabolism and the Apa I polymorphism in IGF-II gene.

Methods: A total of 300 children were selected randomly from the Macrosomia Birth Cohort in Wuxi, China. The height and weight were measured and blood samples were collected. Plasma concentrations of 8 biochemical markers were detected. Apa I polymorphism was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: Biochemical markers were detected for 296 subjects and 271 subjects were genotyped for the Apa I polymorphism. No association was found between birth weight and 8 biochemical markers. In boys, the BMIs of AA, AG and GG genotypes were 16.10 ± 2.24 kg/m^2, 17.40 ± 3.20 kg/m^2, 17.65 ± 2.66 kg/m^2. And there was statistical difference among the three genotypes. But in girls, there was no statistical difference. The birth weights of AA, AG and GG genotypes were 3751.13 ± 492.43 g, 3734.00 ± 456.88 g, 3782.00 ± 461.78 g. And there was no statistical difference among the three genotypes.

Conclusion: Biochemical markers are not associated with birth weight. Apa I polymorphism may be related to childhood BMI, but it may be not associated with birth weight. Therefore, biochemical markers and Apa I polymorphism might not play a role in the association of birth weight and BMI.

Keywords: Biochemical marker, Apa I polymorphism, Birth weight, BMI, IGF-II gene

Introduction

Obesity is a growing global public health issue that currently affects a large part of the world's population irrespective of age, gender or ethnicity (1). Globally, in 2010, the number of overweight and obese children under the age of five was estimated to be over 42 million with about 35 million living in developing countries (2). Obesity is a complicated condition and currently there is no clear-cut comprehensive understanding of its determinants. A clearer understanding of the determinants of obesity can contribute to improve management and prevention measures and to con-

trol the "epidemic" of obesity. Because obesity plays an important role in the pathogenesis of type I diabetes mellitus (3), hypertension (4), cardiovascular disease (5) and some cancers (6, 7), controlling the epidemic of obesity has huge public health significance.

Body mass index (BMI), an index commonly used to measure overweight and obesity, is determined by both genetic and environmental factors. Based on recent evidence, early-life experiences in utero may induce permanent changes in physiologic function programming the long-term regulation of energy balance. This subsequently may affect obesity risk in the later life. Birth weight, which is frequently used as an indicator of the conditions experienced in utero (8), has been related to BMI in childhood (9, 10). Nevertheless, the mechanisms underlying the association are poorly understood. Resetting of major hormonal axes controlling growth and development may be an important mechanism underpinning the association (11, 12). Environmental exposures in prenatal life may imprint the hypothalamic-pituitary-adrenal axis (HPAA) that regulates energy homeostasis, resulting in permanent modification of the neuroendocrine response to stress throughout life (11). Men with low birth weight have increased activity of the HPAA (13). Increased HPAA activity may underlie the development of the obesity. Perhaps the stress resulting from early-life experiences in utero establishes a life-long irreversible lipoprotein profile driven by chronic activation of the HPAA. In addition, long-term effects of experiences in utero on the development of the endocrine pancreas had been observed. "The adverse effect of intrauterine growth restriction on glucose homeostasis was mediated through programming of the fetal endocrine pancreas" (12). Biochemical markers (particularly insulin) represent key signals that program CNS (hypothalamic) and endocrine pancreas development and function and exert lasting effects on body weight regulation and glucose homeostasis (14). So they have been used to research the programming hypothesis of hormonal axes. However, the results are less consistent (15-17). Cholesterol, triglyceride, HDL, apoprotein A1, apoprotein B, glucose, insulin and C peptide are related to major hormonal axes that regulate energy homeostasis and obesity. In the study, their relationships with birth weight were assessed to explore whether they play a role in the association of birth weight with later BMI.

Whereas fetal "programming" may reset the major hormonal axes controlling growth and development, some studies suggest that birth weight and BMI may partly share a common genetic background (18, 19). IGF-I and IGF-are peptide hormones that play an important role in the regulation of metabolism and growth (20). Although IGF-Ⅱ is known to play a key role in fetal growth and development (21), some studies suggest that this hormone may be associated with lipid metabolism and body weight regulation in postnatal life (22). Observational and experimental investigations in humans have shown that a number of allelic variants within the IGF-Ⅱ gene influence body w eight and BMI (23, 24). The Apa I polymorphism in the IGF-Ⅱ gene was also associated with increased adiposity and related metabolic changes in an overfeeding study (25).

We therefore also assessed the associations of the Apa Ⅰ polymorphism in IGF-Ⅱ gene with childhood BMI and birth weight to explore whether the Apa Ⅰ polymorphism in IGF-Ⅱ gene play a role in the association of birth weight with later BMI.

Materials and Methods

Subjects

The subjects came from the Macrosomia Birth Cohort in Wuxi, China, who participated in the US-China collaborative study of periconceptional folic acid supplementation and neural tube defects in the 1990s (26). Detailed characteristics of the birth cohort have been reported previously (27). A total of 300 children were chosen randomly from the cohort whose ages were between 11 and 14 years old. There were 203 boys and 97 girls. The parents who agreed to participate after full explanation of the purposes and procedures of the study were asked to sign consent and to take their children to participate. This study was approved

by the Ethics Committee of Shanghai Institute of Planned Parenthood Research, China.

Methods

A structured questionnaire was used to collect related information. Height and weight were measured by the trained nurses using the standard tools. Birth weight was obtained from the birth cohort records maintained by the local Maternal and Children Health Bureaus. High birth weight (HBW) was defined as birth weight ≥ 4000 g and normal birth weight (NBW) was defined as 2500 g ≤ birth weight < 4000 g (28).

Blood sample was extracted from each selected subject with empty stomach and separated as soon as possible. Plasma concentrations of cholesterol, triglycerides, HDL, apoprotein A1, apoprotein B and glucose were measured using TBA-30FR Automatic Biochemical Analyzer (Toshiba, Tokyo). Plasma concentrations of insulin and C peptide were measured by radioimmunoassay. Insulin resistance index (IRI) was calculated as [plasma glucose (mmol/L)* insulin (mIU/L)] / 22.5. Insulin abnormality was defined as plasma insulin > 19.8 mIU/L and C peptide abnormality was defined as plasma C peptide > 0.60 pmol/ml, while insulin resistance was defined as IRI > 2.8.

DNA was abstracted with Whole Genomic DNA Extraction Kit (Tiangen Biotech CO., Ltd, China). Apa Ⅰ polymorphism (G/A) in the IGF-IIgene, which has been registered in the polymorphic data of Human Gene Mapping 10 as System D of the IGF-Ⅱgene, was detected by PCR-RFLP. The polymorphic Apa Ⅰ site was deduced to be at the 8763-8768th nucleotide in a reported sequence (GenBank X03562) (29). The primers were designed to amplify a 236 bp fragment according to prior report (30). Fw primer: 5'CTT GGA CTT TGA GTC AAA TTG GC3', and Rv primer: 5'GCG GTA CGA GCG ACG TGC CCA C3', were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The IGF-Ⅱgene was amplified by PCR. Then the PCR product was digested using Apa I restriction enzyme. If the Apa Ⅰ polymorphism is GG genotype, Apa I digestion of the PCR product of 236 bp generates two fragments of 63 and 173 bp. If the Apa Ⅰ polymorphism is AG genotype, Apa I digestion of the PCR product generates three fragments of 236, 63 and 173 bp. If the PCR product cannot be digested by Apa I restriction enzyme, the Apa Ⅰ polymorphism is AA genotype.

Categorical variables were summarized as N (percent). Continuous variables were summarized as mean ± standard deviation. T test and analysis of variance was used to test the difference among groups, and Student-Newman-Keuls q test was used in multiple comparisons. Significance was defined as $P<0.05$. All the data analyses were performed with the SAS package (Version 9.0; SAS Institute, Inc., Cary, North Carolina).

Results

Birth weight and the plasma biochemical markers related to metabolism

Biochemical markers related to metabolism, including cholesterol, triglyceride, HDL, apoprotein A1, apoprotein B, glucose, insulin and C peptide, were successfully detected for 296 subjects. According to birth weight, these subjects were divided into two groups: HBW and NBW. No association was found between birth weight and the plasma biochemical markers related to metabolism, the same results were found after adjusting for gender (Table1).

The incidence rates of insulin abnormality, C peptide abnormality and insulin resistance were 9.33%, 12.67%, 30.67% in HBW group, and 6.00%, 10.00%, 23.33% in NBW group. The RRs were 1.24 (95%CI: 0.87, 1.76), 1.13 (95%CI: 0.82, 1.57) and 1.19 (95%CI: 0.94, 1.51), respectively. There was no statistical difference in incidence rates of insulin abnormality, C peptide abnormality and insulin resistance between the two groups.

Apa Ⅰ polymorphism in the IGF-Ⅱ gene and childhood BMI

In the present study, 271 subjects were genotyped for the Apa Ⅰ polymorphism in the IGF-II gene, among whom there were 183 boys and 88 girls. There were 71 subjects exhibiting AA genotype, 150 exhibiting AG genotype and 50 exhibiting GG genotype, accounting for 26.20%, 55.35%

and 18.45% respectively. They were in Hardy-Weinberg equilibrium. The allelic frequencies f(A)=0.54 and f(G)=0.46. The Chi-square test revealed no significant difference between expected and observed frequencies.

Table 1: Birth weight and the biochemical markers related to metabolism

	High birth weight		Normal birth weight					
	Boy(n=100) Mean±SD	Girl(n=50) Mean±SD	Boy(n=100) Mean±SD	Girl(n=46) Mean±SD	$t^{\#}$	$P^{\#}$	$t^{\$}$	$P^{\$}$
Cholesterol (mmol/L)	3.83±0.59	3.86±0.53	3.87±0.57	3.94±0.50	-0.48	0.63	-0.69	0.49
Triglyceride(mmol/L)	0.77±0.32	0.88±0.30	0.78±0.34	0.94±0.33	-0.23	0.82	-1.00	0.32
HDL (mmol/L)	1.56±0.32	1.56±0.34	1.56±0.35	1.57±0.32	-0.14	0.89	0.05	0.96
Apo A1 (g/L)	1.39±0.35	1.41±0.42	1.38±0.22	1.32±0.22	0.40	0.69	1.22	0.23
Apo B (mmol/L)	0.68±0.12	0.70±0.11	0.69±0.13	0.71±0.13	-0.46	0.65	-0.33	0.74
Glucose (mmol/L)	4.72±0.43	4.74±0.43	4.69±0.43	4.72±0.56	0.36	0.72	0.21	0.84
Insulin (mIU/L)	10.98±4.66	13.94±8.38	10.57±6.08	11.60±5.76	0.54	0.59	1.60	0.11
C peptide (pmol/L)	0.35±0.20	0.46±0.27	0.31±0.22	0.41±0.19	1.37	0.17	1.02	0.31
IRI	2.34±1.10	2.99±1.93	2.23±1.33	2.40±1.19	0.63	0.53	1.77	0.08

Note: *t* test was used to test the difference between high birth weight and normal birth weight groups. /#: For boys/$: For girls/High birth weight: Birth weight ≥ 4000 g/Normal birth weight: 2500 g ≤ Birth weight < 4000 g/HDL: High-density lipoprotein/Apo A1: Apoprotein A1/Apo B: Apoprotein B/IRI: Insulin resistance index

The BMIs of AA genotype group, AG genotype group and GG genotype group were 16.41 ± 2.65 kg/m2, 17.11 ± 2.98 kg/m2, 17.50 ± 2.59 kg/m2, respectively. There was no statistical difference in BMI among the three genotype groups with *P*=0.09. In boys, the BMIs of AA genotype group, AG genotype group and GG genotype group were 16.10 ± 2.24 kg/m2, 17.40 ± 3.20 kg/m2, 17.65 ± 2.66 kg/m2, respectively. There was statistical difference in BMI among the three genotype groups with *P*=0.03. Compared with GG boys, AA boys had lower BMI. By multiple comparisons, there was statistical difference in BMI between the AA genotype group and AG genotype groups, and the same result was found between the AA genotype group and GG genotype group, but there was no statistical difference between the AG genotype group and GG genotype group. In girls, there was no statistical difference in BMI among the three genotype groups with *P*=0.70 (Table 2).

Because birth weight, gender and age may be related to the childhood BMI (31), covariance model was used to adjust for these potential factors.

Table 2: Comparison of BMI among different genotype groups (kg/m2)

	N	Mean±SD	AA Mean±SD	AG Mean±SD	GG Mean±SD	*F*	*P*
Boy	183	17.16±2.95	16.10±2.24	17.40±3.20	17.65±2.66	3.60	0.03
Girl	88	16.65±2.58	16.75±3.15	16.43±2.25	17.09±2.37	0.36	0.70
Sum	271	17.00±2.84	16.41±2.65	17.11±2.98	17.50±2.59	2.43	0.09

Note: Analysis of variance was used to test the difference among different genotype groups, and Student-Newman-Keuls q test was used in multiple comparisons

Table 3 shows the result of covariance analysis of BMI among different genotype groups, which indicates that Apa Ⅰ polymorphism, birth weight and age might influence the childhood BMI. There was statistical difference in BMI between the AA genotype group and GG genotype group, but there was no statistical difference between the AG genotype group and GG genotype group.

The incidence rates of overweight/obesity in the three-genotype groups were compared. Overweight and obesity were defined as BMI higher than the sex-age-specific criteria set by the working group on obesity in China (32). The incidence rates of overweight/obesity in the AA genotype group, AG genotype group and GG genotype group were 4.23%, 10.00% and 14.00%, respectively. However, there was no statistical significance with X^2=3.59 and P=0.17.

Apa Ⅰ polymorphism in the IGF-II gene and birth weight

Table 4 presents the comparative result of birth weight among different genotype groups. The birth weight of AA, AG and GG genotype groups were 3751.13 ± 492.43 g, 3734.00 ± 456.88 g, 3782.00 ± 461.78, respectively. No statistical difference was found in birth weight among different genotype groups (P = 0.82). According to birth weight, these subjects were divided into two groups: HBW and NBW. Table 5 shows the distribution of HBW and NBW among different genotype groups after stratifying by gender, and no statistical significance was found (P > 0.05).

Table 3: Covariance analysis of BMI among different genotype groups

	Reference		Parameter estimation	*t*	*P*
Genotype	GG	AG	-0.26±0.45	-0.58	0.56
		AA	-1.10±0.52	-2.14	0.03
Concomitant variable					
Birth weight (g)	Continuous variable		0.92±0.36	2.55	0.01
Age (year)	Continuous variable		0.54±0.23	2.32	0.02
Gender	Girl	Boy	-0.53±0.36	-1.45	0.15

Note: Covariance model was used to adjust for birth weight, age and gender

Table 4: Comparison of birth weight (g) among different genotype groups

	N	Mean±SD	AA Mean±SD	AG Mean±SD	GG Mean±SD	*F*	*P*
Boy	183	3747.16±453.61	3783.66±413.01	3707.68±475.07	3823.61±430.69	1.06	0.35
Girl	88	3747.73±493.22	3718.33±565.91	3805.68±425.93	3628.57±533.75	0.76	0.47
Sum	271	3747.34±465.92	3751.13±492.43	3734.00±456.88	3782.00±461.78	0.20	0.82

Note: Analysis of variance was used to test the difference among different genotype groups, and Student-Newman-Keuls q test was used in multiple comparisons

Table 5: Distribution of stratified birth weight among different genotype groups after stratifying by gender

	Boy (n=183)				Girl (n=88)			
	HBW		NBW		HBW		NBW	
	n	%	n	%	n	%	n	%
AA	22	24.44	19	20.43	16	34.04	14	34.15
AG	51	56.67	55	59.14	24	51.06	20	48.78
GG	17	18.89	19	20.43	7	14.89	7	17.07
Sum	90	100.00	93	100.00	47	100.00	41	100.00
X^2	0.43				0.09			
P	0.81				0.96			

Note: X^2 test was used to test the distributional difference of HBW and NBW among different genotype groups/HBW: High birth weight, birth weight ≥ 4000 g/NBW: Normal birth weight, 2500 g ≤ birth weight < 4000 g

The results above indicate that ApaI polymorphism in IGF-II gene may be not associated with birth weight, and it might not play a role in the association of birth weight with later BMI.

Discussion

Obesity is always accompanied with the abnormality of the metabolism of cholesterol, triglyceride, insulin and so on. Biochemical markers related to metabolism may be related to birth weight which has been associated with obesity (9, 10), and play a role in the association. It was found that the HBW children had a lower hepatic insulin resistance index, lower insulin and free fatty acid concentrations than NBW children (33). HBW was associated with hyperinsulinemia and elevated HOMA-IR (34). The association of birth weight with biochemical markers related to metabolism was not found (35). The similar resluts were found in our study. It could not hypothesize that these biochemical markers play a role in the association of birth weight with later obesity. Those with HBW are more obese but less insulin resistant relative to their body size (36). It is still controversial in the association between birth weight and biochemical markers related to metabolism.

IGF-Ⅱ, also known as somatomedin A, is one of the major determinants of fetal and postnatal growth (37). Gene targeting experiments conducted on mice have demonstrated that inheritance of a null allele of the IGF-Ⅱgene from the father results in a pup considerably smaller than its littermates, whereas maternal inheritance does not result in growth deficiency (38). A study using pigs as an experimental model has detected an influence of the IGF-Ⅱgene on fat deposition (39). Apa Ⅰ polymorphism (G/A) of IGF-Ⅱgene is mapped to an untranslated region (3`UTR) of exon 9. In the present study, AA genotype, AG genotype and GG genotype accounted for 26.20%, 55.35% and 18.45% respectively. The allelic frequencies f (A)=0.54 and f(G)=0.46. Compared with the findings from previous report (40), a high frequency of AA and a low frequency of GG were observed in the current study.

To date the literature data, concerning the relationship of the Apa Ⅰ polymorphism with BMI, remains controversial. In a study on 1474 healthy Caucasoid men aged 45-65y, the IGF-Ⅱ Apa I AA homozygote showed a mean body weight 4 kg lower than the Apa II GG homozygote (77.6±10.9 kg vs 81.6±11.5 kg, P=0.003) with heterozygote (GA) intermediate (80.1±11.9 kg). The IGF-Ⅱ Apal AA genotype was therefore associated with lower mean body weight than the GG genotype. Apa ⅠGG homozygote incurred a 1.67-fold risk of pathological BMI (> 30 kg/m^2) compared with AA homozygote (41). Roth et al have found that individuals homozygous for the IGF-Ⅱ G allele do not exhibit higher body mass, BMI or fat mass compared to AA individuals and that Caucasians with the AA genotype exhibit higher fat mass than GG individuals (42). In our study, there was statistical difference in BMI of boys among the three-genotype groups (AA genotype group, AG genotype group and GG genotype group). Compared with GG boys, AA boys had lower BMI. By multiple comparisons, there was statistical difference in BMI between the AA and AG genotype groups, and the same result was found between the AA and GG genotype groups, but there was no statistical difference between the AG and GG genotype groups. In girls, there was no statistical difference in BMI among the three-genotype groups. Therefore, we hypothesize that the association of Apa Ⅰ polymorphism in the IGF-Ⅱgene with childhood BMI may be influenced by gender. However, it may also be the case that we did not found the association in girls because the sample only included a small number of girls.

Birth weight shows a positive association with childhood BMI (9, 10). The mechanisms underlying the association are poorly understood to date. Genetic factors contribute to variation in both birth weight and BMI. The heritability estimates range between 20% and 50% for birth weight (43, 44) and between 40% and 70% for BMI (45, 46). Some studies suggest that birth weight and BMI may partly share a common genetic background (18, 19). Kilpelainen et al. examined the associa-

tions of 12 established BMI variants and their additive score with birth weight. Only the MTCH2 and FTO loci showed a nominally significant association with birth weight. The BMI-increasing allele of the MTCH2 variant (rs10838738) was associated with a lower birth weight (β ± SE: -13 ± 5 g/allele; $P = 0.012$; n = 23,680), and the BMI-increasing allele of the FTO variant (rs1121980) was associated with a higher birth weight (β ± SE: 11 ± 4 g/allele; $P = 0.013$; n = 28,219). These results were not significant after correction for multiple testing. Therefore, they suggest obesity-susceptibility loci have a small or no effect on weight at birth (47). In our study, we examined the association of Apa I polymorphism in IGF-II gene related to BMI with birth weight. It was found that the Apa I polymorphism was not related to birth weight. We hypothesize that the Apa I polymorphism in the IGF-II gene does not explain the association between birth weight and BMI in childhood. However, a study performed by Kaku et al found that there was a significant difference in birth weight SDSs among the three genotypes (48). Our results are partly consistent with those of the study performed by Gomes et al. (40). Gomes et al tested the association between IGF-II ApaI genotype and BMI/birth weight in 294 healthy volunteers. Although they did not found that the IGF-II genotype was significantly associated with BMI and/or birth weight, they observed a statistically significant correlation of 0.33 ($P < 0.023$) between birth weight and BMI in GG subjects whose birth weight was higher than 3.5 kg (n = 47). Therefore, they hypothesize that HBW associated with homozygosis for the G allele is not a null factor and might be associated with predisposition to high BMI in young adults (40).

In the study, we explored the mechanisms underlying the association of birth weight with later BMI from the biochemical markers related to metabolism and the Apa I polymorphism in IGF-II gene. Nevertheless, some limitations need to be addressed. One limitation of our data was the small sample size. Finally, the lack of information on additional characteristics, such as eating and physical activity habits, is also a limitation of this analysis.

Conclusion

Biochemical markers related to metabolism are not associated with birth weight. Apa I polymorphism in IGF-II gene may be related to childhood BMI, but it may be not associated with birth weight. Therefore, biochemical markers related to metabolism and Apa I polymorphism in IGF-II gene might not play a role in the association of birth weight and BMI.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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