Early Human Development 90 (2014) 545–548

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Association of the intronic polymorphism rs12540874 A>G of the GRB10 gene with high birth weight

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ARTICLE INFO

Article history:
Received 11 January 2014
Received in revised form 18 June 2014
Accepted 24 June 2014

Keywords:
GRB10
Polymorphism
High birth weight

ABSTRACT

Background: High birth weight (HBW) is considered a key predictor of the development of chronic diseases, such as Type 2 Diabetes (T2D). Foetal growth depends on many factors, among which placental function is critical. Some genes with expression in the placenta, such as *GRB10*, are known to be involved in the regulation of insulin receptor pathways and the size of mouse littermates.
Aim: To evaluate whether the intronic polymorphism rs12540874 A>G of the *GRB10* gene is associated with HBW in term newborns.
Study design: A total of 51 healthy term newborns were enrolled in a nested case–control study. The case group was defined by the presence of HBW (n = 17) and the control group by newborns with normal birth weight (NBW n = 34). Maternal and foetal factors influencing HBW were considered as exclusion criteria. The polymorphism was determined through real-time PCR using TaqMan technology. Categorical variables were evaluated with descriptive statistics, and multivariate logistic regression analysis was used to evaluate the association between polymorphism and HBW.
Results: The newborns in the case group had a longer gestation period (39.7 ± 1.0 and 38.8 ± 1.8 weeks) and higher insulin levels at birth (9.5 ± 4.0 and 5.7 ± 3.4 μU/mL) than the newborns in the control group. The multivariate regression analysis, adjusted for weeks of gestation, showed a significant association between the SNP rs12540874 A>G of the *GRB10* gene with HBW (OR 4.9; $CI_{95\%}$ 1.10–22.10 $p = 0.02$).
Conclusions: Our results suggest that the SNP rs12540874 A>G, an intronic SNP of the gene *GRB10*, is associated with HBW.

1. Introduction

A growing body of evidence shows that alterations of birth weight (BW) increase the risk of disease later in life through a phenomenon termed "early life programming" [1].

A complex interaction between environmental, psychological, sociological, hormonal, maternal, paternal, foetal, genetic, and placental factors determines BW [2], and mismatches in these interactions can lead to abnormal foetal BW [3].

The placenta plays a regulatory role in the exchange of waste and nutrients between the mother and foetus. During foetal life, several genes expressed in the placenta play an important role in the control of resource utilisation; thus, the single nucleotide polymorphisms (SNPs) in these genes may affect placental function and thereby may influence the growth and development of the foetus [4].

One of genes involved in the appropriate foetal and placental growth is the growth factor receptor-bound protein 10 (GRB10) [5]; this gene is subjected to imprinting and its expression is determined by the maternal allele [5]. The *GRB10* gene acts on insulin receptor signalling pathway [6], a well-known mechanism related to BW [7].

At least seven alternative variants of the *GRB10* gene have been identified; of these, the hGrb10α and hGrb10β have intermediated expression in the placenta [8]. The *GRB10* gene is located on 7p12.2 and contains 17 exons and 2789 SNPs [6]; of those, the intronic SNP rs12540874 A>G was found to be positively associated with human

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http://dx.doi.org/10.1016/j.earlhumdev.2014.06.012
0378-3782/

height in the population-based FINRISK97 cohort (n = 7803) [9]. In this context, the objective of this study was to determine whether polymorphism rs12540874 of the *GRB10* gene is associated with HBW.

2. Material and methods

The Mexican Social Security Institute Ethics Committee, in accordance with The Code of Ethics of the Declaration of Helsinki, approved the study; the mother's informed consent, which included approval for prospective use of data, was obtained.

We conducted a nested case–control study in which 51 placental samples, formalin-fixed and paraffin embedded (FFPE), were analysed. Samples were obtained from mothers of healthy term newborns with HBW and newborns with NBW who were enrolled in a previous cohort study conducted by our group [10].

The case group, defined by the presence of HBW at birth, was compared with a control group of newborns with NBW. The inclusion and exclusion criteria have been reported elsewhere [10]. In brief, newborns delivered from healthy mothers without a diagnosis of diabetes, gestational diabetes, history of previous gestational diabetes, high blood pressure, or malnutrition were enrolled in a follow-up study. Preterm delivery, intrauterine infection and congenital malformations were exclusion criteria.

Some characteristics of the mothers, such as age, smoking, alcohol intake, number of gestations, weight and body mass index (BMI) in the previous pregnancy, as well as weight gain in the current pregnancy were matched criteria.

The health status of the mothers was confirmed by direct detailed medical history, physical examination and review of the medical records of their prenatal control.

A total of 300 newborns with gestational ages between 38 to 41 weeks were enrolled in the original cohort; of these, 17 (5.7%) newborns with HBW were assigned to the case group and compared with a control group of 34 (11.3%) newborns with NBW.

The DNA was obtained from FFPE placental samples for identification of intronic SNP rs12540874 A>G.

2.1. Definitions

HBW was defined as a BW in a term newborn ≥4000 g and NBW as a BW ≥2500 and <4000 g [11].

Hyperinsulinemia was defined as having insulin levels ≥5 μU/mL [12].

2.2. Measurements

The BW was obtained within the first hour of the newborn's life and a blood sample was taken from the vein cord within 3 min after delivery [10].

The maternal weight and height from the previous pregnancy were obtained from medical records; BMI was calculated according to the following formula: BMI = Weight (kg) / Height $(m)^2$ [13].

The FFPE blocks of the placenta were cut with a microtome (American Optical® Buffalo, NY) to obtain 7 slices of 20 μM each. The DNA was extracted with a commercial kit NucleoSpin® FFPE DNA distributed by Macherey-Nägel following the manufacturer's instructions with slight modifications (longer de-paraffinisation time); briefly, the procedure consisted of paraffin removal, sample lysis, decrosslinking of DNA, binding DNA to the NucleoSpin® column and DNA elution. The DNA integrity was verified through 1% agarose gel electrophoresis and purity and concentration by spectrophotometry at 260/280 nm in a Nanodrop 2000c equipment (ThermoScientific®).

Genotyping was performed with a Real Time PCR system StepOne™ (Applied Biosystems®) using TaqMan technology. A total of 25 ng of genomic DNA was used under the following reaction conditions: one cycle of initial denaturing at 95 °C/10 min followed by 42 cycles of denaturing (95 °C/15 s), annealing (60 °C/1 min) and extension (60 °C/30 s). The intronic SNP rs12540874 A>G was recognised with the TaqMan® MGB Probe (C_11450004_10).

The sample power was calculated contrasting 17 subjects in the first group and 34 in the second, based on an alpha two-tailed value of 0.05; the power of the hypothesis test was 65%. [14].

2.3. Statistical analysis

Data are presented as the mean ± standard deviation or proportions. Differences between numerical variables were established with Student's *t* test for independent samples (Mann Whitney *U* for non-parametric data) and the X^2 (*Fisher's exact test*) test for categorical variables.

The frequencies of genotypes were obtained by direct count, and Hardy–Weinberg Equilibrium (HWE) was calculated through the X^2 goodness-of–fit statistic; both analyses were carried out by using the program SNPstats (http://bioinfo.iconcologia.net/SNPstats). The association between polymorphism and HBW was evaluated with multivariate logistic regression analysis. The model was adjusted by the variables from the bivariate analysis that showed a significant between-group difference.

Statistical significance was set at $p < 0.05$ with a 95% confidence interval (CI). Statistical analysis was performed using SPSS V.15.0.

3. Results

A total of 51 healthy newborns were enrolled in the study; of these, 17 (33.3%) newborns with HBW and 34 with NBW (66.7%) were allocated to the case and control groups, respectively.

Newborns in the case group had a longer gestation period and higher insulin levels at birth, than newborns in the control group; there were no significant differences for the other variables, Table 1.

The allele and genotype frequencies of SNPs rs12540874 A>G of the *GRB10* gene are shown in Table 2. There were no significant differences in the allele ($X^2 = 2.2$, $p = 0.14$) and genotype frequencies between the case and control groups for the SNP rs12540874 A>G.

The population in the control group was in HWE and the association of SNP rs12540874 A>G of the *GRB10* gene with HBW agrees with a dominant inheritance model.

The crude multivariate regression analysis showed that the SNP rs12540874 A>G of the *GRB10* gene was significantly associated with HBW (OR 4.1; $CI_{95\%}$ 1.01–17.1, $p = 0.03$); in the multivariate analysis adjusted by weeks of gestation, the SNP rs12540874 A>G of the *GRB10* gene remained significantly associated with HBW (OR 4.9; $CI_{95\%}$ 1.10–22.10, $p = 0.024$).

Table 1
Characteristics of mothers and newborns according to birth-weight at birth.

	Case	Control	
N	19	34	*P* value
Age, y	26.3 ± 4.3	25.3 ± 4.5	0.46
Alcohol intake, n (%)	1 (5.3)	3 (8.8)	0.073
Smoking, n (%)	1 (5.3)	5 (14.7)	0.038
Number of gestations	2.3 ± 0.9	2.4 ± 1.2	0.94
Weight previous pregnancy, kg[a]	65.8 ± 8.1	60.1 ± 12.1	0.052
Body mass index, previous pregnancy*	24.9 ± 3.7	23.4 ± 4.4	0.21
Weight gain during pregnancy, kg	14.9 ± 3.4	12.9 ± 5.6	0.12
Weeks of gestation	39.7 ± 1.0	38.5 ± 2.4	0.019
Weight of newborn, g	4156 ± 184	2910 ± 599	<0.0005
Glucose levels at birth, mg/dL	101.6 (73–109)	81.1 (68–89)	0.088
Insulin at birth, μU/mL	9.5 ± 4.0	5.7 ± 3.4	0.001

* = N; ** = Fisher´s exact test; † = chi-square test.
[a] Values are mean ± SD, otherwise is indicated.

Table 2
Allele and genotype frequencies for SNP rs12540874 for the gene *GRB10*.

SNP 12540874			
	Case	Control	
	N = 17*	N = 35	*p* value
A	15(44)	43(61)	0.14
G	19(56)	27(39)	
AA	3(17.6)	16(45.7)	0.06*
AG	9(53)	11(31.4)	0.23**
GG	5(29.4)	8(23)*	0.73*

*N = Individuals; values are n (%).
*Fisher's exact test.
** $X^2 = 1.42$, $p = 0.23$.

4. Discussion

Our results suggest that the SNP rs12540874 A>G, an intronic SNP of the gene *GRB10*, is associated with HBW.

It is well known that HBW is associated with metabolic syndrome in childhood [15], particularly in the presence of maternal obesity and gestational diabetes [16].

Foetal growth is largely determined by the supply of nutrients across the placenta [17]. Experimental studies in mice show that among the genes with placental expression, the *Grb10* gene is expressed in the labyrinthine compartment of the placenta, the site for the exchanging of nutrients between the maternal and foetal compartments; *Grb10* also regulates foetal and placental development through activated receptors in pathways that involve *RAF1* and protein kinase Ba [18]. It has been postulated that imprinted genes mediate a network of supply and demand factors that determine foetal growth [19]. In this regard, the *Grb10* gene expressed from the maternally inherited chromosome may modulate placental growth in response to a foetal demand signal [20]. As a result of deleting both copies of the *Grb10 gene*, mouse littermates show overgrowth [7]. In humans, the normal functions of the *GRB10 gene* counteract growth-promoting factors in the foetus [20]; the presence of two maternal copies of the *GRB10* gene determines the presence of growth retardation Silver–Russell syndrome [21].

The *GRB10* gene is a well-known negative modulator of insulin receptor function, as it competes for the same domains of insulin receptor with insulin receptor substrate 1, inhibiting the downstream P13K pathway [6]. Disruption of the *GRB10* gene on peripheral tissues leads to enhanced insulin signalling, [22] while in mouse models, the overexpression of the *Grb10* gene leads to an insulin resistance phenotype [23]. Therefore, an increase in the expression of the *GRB10* gene is expected to generate an "insulin resistance like" phenotype, which could explain the observed hyperinsulinemia in HBW newborns. Further research in the field is necessary to confirm this hypothesis.

Nonetheless, that prolonged gestation is related to an increase in BW, the difference in gestational age between the groups in the study do not explain, per se, the increased BW of the newborns in the case group. In this regard, during the last month of gestation, the expected weight gain per week is approximately 200 g [24]. Thus, the observed differences in weeks of gestation (Table 1) do not explain the differences in BW.

The intronic nature of the analysed polymorphism suggests that it may decrease the gene expression of *GRB10* by acting as an active site for pre-mRNA splicing [25,26], or even as a potential modifier of either methylation [27] or histone sites [28].

It has been previously reported that there is an association between the synonymous exonic polymorphism rs1800504 of the *GRB10* gene and birth size [29], but unlike our study, this association was non-significant.

Some polymorphisms of the *GRB10* gene (rs2237457, rs2190496, rs2237478 and rs7805310) have been associated with type 2 diabetes [30]; thus, further research is necessary to clarify the functional consequences of SNP rs12540874 A>G of the *GRB10* gene among individuals with diabetes as well as to clarify the role of its expression as a predictor of hyperglycemia.

There are several limitations to this study. First, given the design of the study, it was not possible to determine the parental origin of the alleles or to analyse samples from the siblings of participants. Second, given the small sample size in this study, our results should be considered as preliminary, and further research in the field is necessary in order to confirm our findings.

In conclusion, the results of this study suggest that the presence of rs12540874 A>G of the *GRB10* gene is associated with HBW.

Conflict of interest statement

The authors state that they do not have any conflict of interest. The manuscript was written by the main author. No honorarium, grant, or other form of payment was given to anyone to produce the manuscript.

Acknowledgement

We thank the physicians and nursing personnel of the participating hospitals that provided the facilities to carry out the clinical work. Funding was provided by the Secretaría de Investigación y Posgrado (SIP)-IPN with the project 20131755. This work also received partial financial support from foundation, IMSS A.C.

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