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The growth hormone receptor exon 3 polymorphism is not associated with height or metabolic traits in healthy young adults

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ABSTRACT

Context: The *GHR* polymorphisms contribution to the interindividual variability in prenatal and postnatal growth as well as to metabolic traits is controversial.
Objective: The aim of this study is to analyze the association of the *GHRfl/d3* polymorphism with prenatal and postnatal growth and metabolic outcomes in adult life and to compare the genotype distribution in different populations.
Design: 385 community healthy subjects followed from birth to adult life (23–25 years old) were grouped according to birth size: small—SGA ($n = 130$, 62 males), appropriate—AGA ($n = 162$, 75 males) and large for gestational age—LGA ($n = 93$, 48 males). *GHRfl/d3* genotype distribution and its potential association with anthropometric (at birth, childhood and adult life) and metabolic features (in adult life) were analyzed and compared with data obtained from a systematic review of *GHRfl/d3* association studies (31 articles).
Results: The frequency of the *GHR d3/d3* genotype was lower in the LGA ($\chi 2$ $p = 0.01$); SGA and AGA subjects exhibited an increased chance of the *d3/d3* genotype (OR = 3.58; 95%CI: 1.55; 8.24) and (OR = 2.39; 95%CI: 1.02; 5.62), respectively. Despite the different prevalence among different birth size groups, in adults, *GHRfl/d3* genotype was not associated with height, plasma IGF1 levels or metabolic phenotype and cardiovascular risk. *GHRfl/d3* genotype distributions in AGA, SGA and LGA groups were comparable with those found in subjects of European origin but not with those of Asian ancestry.
Conclusions: The *GHRd3* genotype was negatively associated with birth size but it was not associated with adult height or weight, plasma IGF1, metabolic phenotype or any marker of increased cardiovascular risk in young adults.

1. Introduction

The growth hormone receptor (GHR) is a cell membrane molecule that belongs to the cytokine receptor superfamily and mediates the majority of growth hormone (GH) actions. Its importance in the growth regulation was demonstrated in Laron syndrome, a short stature condition caused by *GHR* mutations [1].

The *GHR* alleles determine isoforms differing by the maintenance (GHRfl—full length) or exclusion (GHRd3—exon 3 deleted) of exon 3 [2]. *In vitro*, GH-binding properties of the GHRfl and GHRd3 isoforms are similar but GHRd3 induces higher transcriptional activity [3]. *In vivo*, some studies have shown that the GHRd3-isoform appears to increase the responsiveness to recombinant human growth hormone (rhGH) replacement therapy in SGA individuals [3], in patients with GH deficiency [4] and in patients with Turner syndrome [5]. However, in GH deficient patients, this effect was observed mainly in the beginning of the treatment, not in final height [4]. In addition, one Spanish study evaluating SGA children suggested that the d3/fl genotype did not influence the response to GH therapy [6]. Therefore, this issue is still a matter of debate and a recent meta-analysis concluded that *GHR* genotypes account only modestly for increases in rhGH effects [7].

Whether the *GHRfl/d3* polymorphism contributes to the interindividual variability of growth in normal subjects is still a matter of debate. A previous association study showed significant differences in the *GHR* genotype distribution in individuals born SGA compared to AGA. This data suggested that GHR isoforms might account for variation in prenatal growth [8].

Moreover, birth size has been associated with adult height as well as with metabolic phenotype in adults. Most studies have shown that both SGA and LGA individuals are at increased cardiovascular risk than AGA [9,10]. Therefore, as the *GHRfl/d3* polymorphism is potentially involved

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in prenatal and in postnatal growth, it is possible that this common genetic variant influences metabolic risk in adult life.

In this study, we have examined the frequency distribution of *GHRfl*/*d3* polymorphism in a cohort of SGA, AGA and LGA subjects followed from birth to adult life. We have also compared the genotype frequency in each group with those found in previous published series and investigated whether *GHR* genotype might be associated with birth size, postnatal growth, and metabolic and cardiovascular risk in early adulthood.

2. Subjects and methods

2.1. Subjects

Our sample is part of a prospective cohort from Ribeirao Preto, Sao Paulo, Brazil. The subjects of this cohort were evaluated in several phases (birth, early childhood and early adulthood). In the first phase (1978–1979), 9067 liveborns (98% of all births in the city) were examined. During the follow-up of the cohort, 6973 liveborns remained in the study. At 23–25 years of age (2001–2003), 2063 were evaluated to analyze the influence of early events on chronic diseases in adult life. Of these 2063 subjects, a subset of about 60% had been evaluated at 9–10 years to analyze childhood growth [11]. The participants of this prospective cohort were classified into birth size groups SGA ($N = 201$), AGA ($N = 1736$) and LGA ($N = 126$). In the present study, subjects enrolled were elected by random selection among each birth size group (see Fig. 1).

We evaluated 385 young subjects, distributed into three groups according to birth size: SGA ($n = 130$, 62 males), AGA ($n = 162$, 75 males) and LGA ($n = 93$, 48 males). Birth size was classified using the references by Willams *et al.*, 1982 [12], for gestational age and sex, as previously described [10]. LGA infants were defined as having neonatal weight above the 90th percentile, SGA children with neonatal weight under the 10th percentile and the AGA children with neonatal weight between the 10th and 90th percentiles.

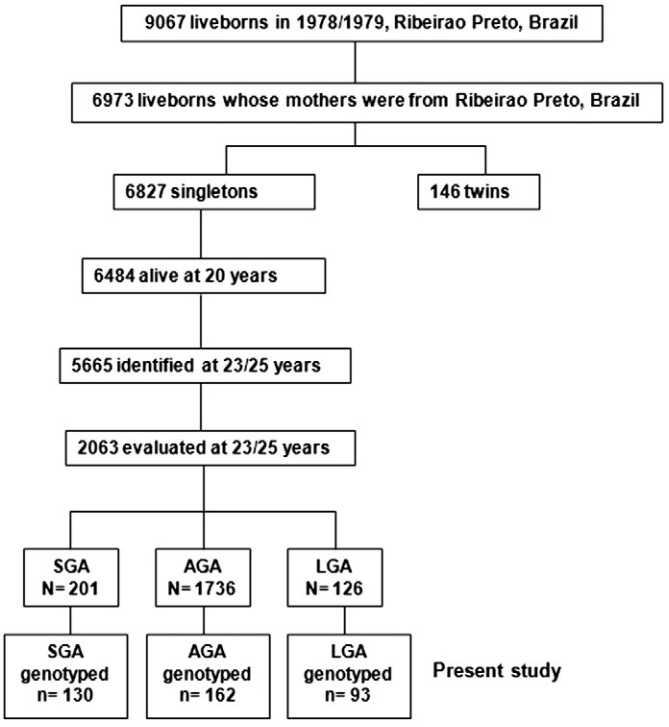

Fig. 1. Characteristics of liveborn individuals of the 1978/79 population-based prospective cohort from Ribeirao Preto, Sao Paulo, Brazil, from which our sample was originated. Birth size groups: small (SGA), appropriate (AGA) and large (LGA) for gestational age.

The exclusion criteria were clinical history of perinatal injury (hypoxic encephalopathy, sepsis, bronchopulmonary dysplasia); metabolic, genetic, pituitary, bone and muscle diseases or any other disease capable of prejudicing growth; disorders affecting serum levels of IGF1 (chronic or acute liver disease, diabetes, malnutrition, anorexia, renal failure defined as serum creatinine values above 106.08 μmol/l in women and above 114.92 μmol/l in men) or use of drugs that may disturb somatotrophic axis including glucocorticoids, estrogens, androgens, anticonvulsants, and GH.

2.2. Data basis

The clinical and biochemical data of the subjects of the present cohort study were obtained from data basis, as follows:

- Perinatal data: birth size, gestational age at birth, cesarean or vaginal birth, perinatal injury, and congenital or acquired disease.
- At 9–10 years old: height, weight, and body mass index (BMI).
- At 23–25 years old: presence of chronic diseases, chronic use of drugs, height, weight, BMI, abdominal and hip circumferences, arterial blood pressure, and biochemical dosages.

All the anthropometric (height SDS—standard deviation score, BMI, waist circumference) and the clinical measurements were performed using standard techniques. Birth weight and length SDS adjusted for sex and chronological age were calculated according to Usher and Mc Lean standards [13], as previously described [10], using Growth Analyzer software (http://www.Growthanalyser.org). Anthropometric SDS for sex and chronological age at 9–10 and 23–25 years were calculated using the references of the Center for Disease Control and Prevention (http://www.cdc.gov). Procedures to blood pressure (BP, mm Hg) estimation followed international standards and were reported in details previously [11]. The presence of clinical coronary disease and thrombosis was assessed by self-reporting.

The biochemical measurements had been performed during the development of the cohort study [11] and in a previous study assessing IGF1 and the components of metabolic risk [10]. Biochemical variables included fasting glucose, insulin, Homeostasis Model Assessment—Insulin Resistance (HOMA-IR) [14], quantitative insulin sensitivity check index (QUICKI) [15], high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, IGF1 and fibrinogen levels. Plasma IGF1 levels were measured by RIA (DSL, Inc-5600); intra-assay and inter-assay variations were 3.0% and 10.8%, respectively.

2.3. DNA samples

Genomic DNA was isolated from blood samples, using QIAmp Blood Kit (QIAGEN), according to the manufacturer's instructions.

2.4. Genotyping

The *GHRfl*/*d3* polymorphism was genotyped using an adapted multiplex PCR [2]. The assay was performed with primers G1, G2, and G3 (GenBankTM accession number AF155912), as follows: an initial step of denaturation of 5 min at 94 °C, followed by 37 cycles consisting of 1 min at 94 °C, 30 s at 60 °C, and 1 min and 30 s at 72 °C, followed by an extension period at 72 °C for 10 min. This assay allows the identification of a 935-bp fragment representing the *fl* allele and a 532-bp fragment representing the *d3* allele. Thus, three different patterns of amplification products are possible, each corresponding to a different genotype: *fl*/*fl*, *fl*/*d3* or *d3*/*d3* (see Supplementary Fig. 1). In order to avoid misinterpretation caused by insufficient amplification of the *fl* allele in the multiplex PCR, for all individuals genotyped as *d3*/*d3* a second PCR was performed using the primers G1 and G3, which amplify specifically the *fl* alleles [8,16,17].